Customer No. 25533

US Patent No. 6,020,329
Attorney Docket No. PC9137C

08/958864

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE: U. S. PATENT NO. 6,020,329

ISSUED: FEBRUARY 1, 2000

TO: JOHN HARGREAVES BATESON, ET AL.

FOR: CEPHALOSPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

ASSIGNEE: PFIZER INC.

Transmittal Letter for Application for Extension of Patent Term Under 35 U.S.C. § 156

Mail Stop Hatch-Waxman PTE
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

Transmitted herewith is the application of Pfizer Inc., dated June 20, 2008, for extension of the term of United States Patent No. 6,020,329 under 35 U.S.C. § 156, together with a duplicate of the papers thereof, certified as such.

Please charge the sum of $1,120.00 to Deposit Account No. 16-1445. Please also charge any additional fees which may be required by the filing of this application for Extension of Patent Term, or credit any overpayment, to Deposit Account No. 16-1445. Two copies of this paper are enclosed.

Dated: JUNE 20, 2008

John H. Engelmann
Attorney for Applicant
Registration No. 28,075

Pfizer Inc.
Patent Department
7000 Portage Road (KZO-267-635)
Kalamazoo, Michigan 49001
(269) 833-2532

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IN RE: U. S. PATENT NO. 6,020,329

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600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

Transmitted herewith is the application of Pfizer Inc., dated June 20, 2008, for extension of the term of United States Patent No. 6,020,329 under 35 U.S.C. § 156, which is currently set to expire on July 22, 2011, based on the materials set forth herein and in the accompanying papers. If this extension is granted, such patent would not expire until July 23, 2015. In the materials set forth herein, paragraph numbers correspond to paragraph numbers in 37 C.F.R. § 1.740(a).

(1) The approved product is CONVENIA®. CONVENIA® is presented as a vial of lyphilised powder and a vial of diluent.

POWDER

852 mg cefovecin (as sodium salt)

19.17 mg methyl parahydroxybenzoate

2.13 mg propyl parahydroxybenzoate

DILUENT

13 mg/ml benzyl alcohol

10.8 ml water for injections

RECONSTITUTED SOLUTION

80.0 mg/ml cefovecin (as sodium salt)

1.8 mg/ml methyl parahydroxybenzoate

0.2 mg/ml propyl parahydroxybenzoate

12.3 mg/ml benzyl alcohol

Generic Name. Cefovecin sodium

Chemical Name. (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)(methoxyimino)-acetamido]-8-oxo-3-[tetrahydrofuran-2(S)-yl]-5-thia-1-azabicylo[4.2.0]oct-2-ene-2-carboxylic acid monosodium salt

This compound is also known by alternate chemical names as:

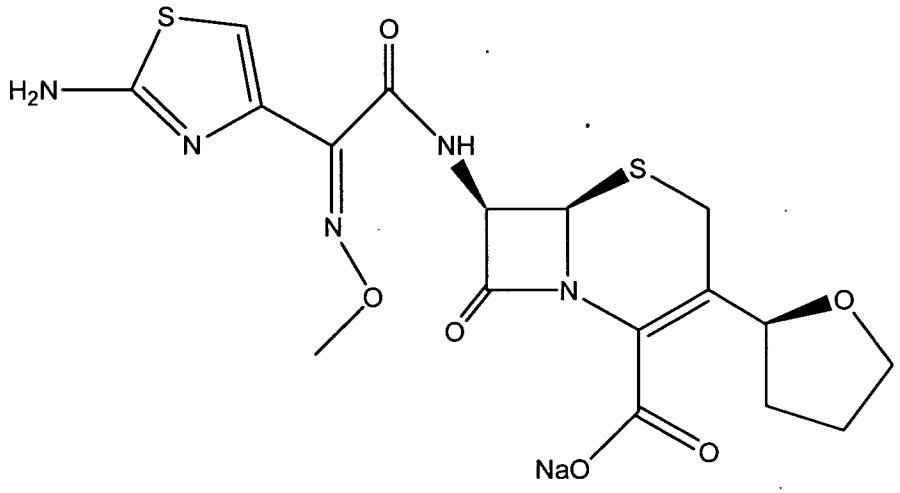
5-thia-1-azabicylo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[{(2Z)-(2-amino-4-thiazolyl)(methoxyimino)acetyl}amino]-8-oxo-3-[(2S)-tetrahydrofuranyl] monosodium salt (6R,7R) and

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

Molecular Formula. $C_{17}H_{18}N_5NaO_6S_2$

Molecular Weight. 475.48

Structural Formula.



(2) CONVENIA® was subject to regulatory review under subsection 512 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 360b) under INAD Application No. 10-612 and INAD Application No. 10-613.

(3) CONVENIA® received permission for commercial marketing or use under subsection 512 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 360b) on April 25, 2008, under New Animal Drug Application (NADA) No. 141-285.

(4) The active ingredient in CONVENIA® is cefovecin sodium. Neither cefovecin sodium nor any other salt of cefovecin has been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act.

(5) This application is being submitted within the sixty day period permitted for its submission pursuant to 37 C.F.R. § 1.720(f). The last day on which this application could be submitted is June 23, 2008.

(6) The patent for which an extension is being sought is identified as follows:
U.S. Patent No.: 6,020,329

Title: CEPHALOSPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

Issued: February 1, 2000

Expires: July 22, 2011

Inventors: John Hargreaves Bateson
George Burton
Stephen Christopher Martin Fell

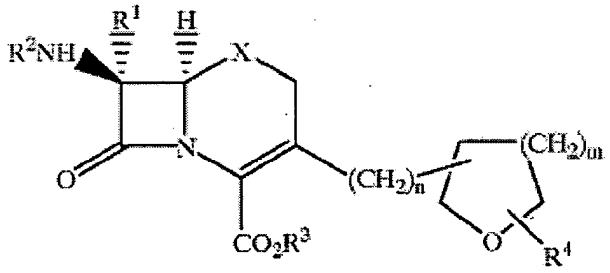
(7) A copy of United States Patent No. 6,020,329, the patent for which an extension is sought, is appended as Exhibit A. On the face of the patent, the assignee reads "Pzifer", which should instead read "Pfizer." A copy of the chain of assignments is appended hereto as Exhibit G.

(8) A certificate of correction or reexamination certificate has issued for United States Patent No. 6,020,329, appended as Exhibit B, and two receipts for maintenance fee payments have issued for this patent. Copies of each receipt are attached hereto as Exhibits C and D.

(9) United States Patent No. 6,020,329 claims the active ingredient of the approved product.

Claim 1 of U.S. Patent 6,020,329 reads
"A compound of formula (I) or a salt thereof:

(I)



wherein R^1 is hydrogen, methoxy or formamido;

R^2 is an acyl group;

CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group;

R^4 represents hydrogen or up to four substituents selected from alkyl, alkenyl, alkynyl, alkoxy, hydroxy, halogen, amino, alkylamino, acylamino, dialkylamino, CO_2R, $CONR_2$, SO_2NR_2 (where R is hydrogen or C_{1-6} alkyl) and aryl, which may be the same or different and wherein any R^4 alkyl substituent is optionally substituted by any other R^4 substituent;

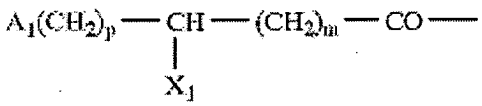
X is S, SO, SO_2, O or CH_2;

m is 1 or 2;

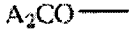
n is 0;

"acyl" is selected from the group consisting of formula (a) to (f):

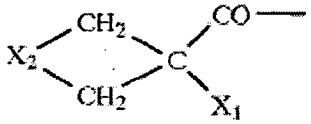
(a)



(b)

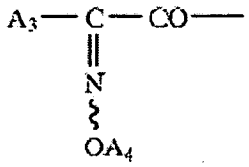


(c)

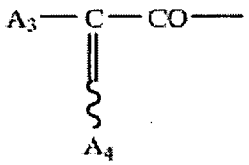


(d)

(e)



(f)



wherein p is 0, 1 or 2;

m is 0, 1 or 2;

A_1 is (C_{1-6})alkyl, substituted (C_{1-6})alkyl, (C_{3-6})cycloalkyl, cyclohexenyl, cyclohexadienyl, or an aromatic group;

X_1 is a hydrogen or halogen atom, a carboxylic acid, carboxylic ester, sulphonic acid, azido, tetrazolyl, hydroxy, acyloxy, amino, ureido, acylamino, heterocyclylamino, guanidino or acylureido group;

A_2 is an aromatic or a substituted alkyl group, or a substituted dithietane;

X_2 is a $-CH_2OCH_2-$, $-CH_2SCH_2-$ or alkylene group;

X_3 is an oxygen or sulphur atom;

A_3 is an aryl or heteroaryl group; and

A_4 is hydrogen, (C_{1-6})alkyl, (C_{3-8})cycloalkyl, (C_{3-8})cycloalkyl(C_{1-6})alkyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, (C_{2-6})alkenyl, carboxy(C_{1-6})alkyl, (C_{2-6})alkynyl, aryl or (C_{1-6})alkyl substituted by up to three aryl groups. "

Claim 1 reads on cefovecin sodium where

R^1 is hydrogen,

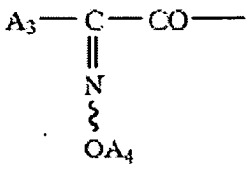
R^2 is an acyl group,

CO_2R^3 is a carboxylate anion,

R^4 is hydrogen,

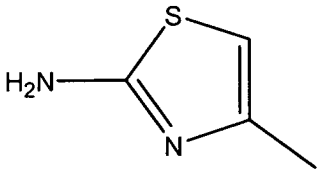
m is 1,

acyl is



A_4 is methyl,

A_3 is a heteroaryl group specifically



Claim 1 therefore claims the active ingredient (cefovecin sodium) of CONVENIA®.

In addition, claims 2, 3, 5, 6, 7 and 8 read on cefovecin sodium. Claim 9 reads on cefovecin sodium because it specifically names the compound.

(10) (A) An exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective for CONVENIA® on November 16, 1999, following submission of Investigational New Animal Drug ("INAD") Application No. 10-612 on August 5, 1999.

(B) An exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective for CONVENIA® on November 16, 1999, following submission of Investigational New Animal Drug ("INAD") Application No. 10-613 on August 6, 1999.

C) A New Animal Drug Application ("NADA") under section 512 of the Federal Food, Drug and Cosmetic Act for CONVENIA® (cefovecin sodium) was initially submitted on March 15, 2008, as NADA No. 141-285.

D) NADA No. 141-285 was approved on April 25, 2008.

(11) A brief description of the significant activities undertaken by or for the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities is attached hereto as Exhibits E and F. Exhibit E is a Chronology of Review of CONVENIA® (Cefovecin sodium) File for Dogs. Exhibit F is a Chronology of Review of CONVENIA® (Cefovecin sodium) File for Cats.

(12) It is Applicant's opinion that United States Patent No. 6,020,329 is eligible for an extension of its term, under 35 U.S.C. §156. The length of the extension claimed is 1462 days. If this extension is granted, the term of such patent, which is currently set to expire on July 22, 2011 (see paragraph 8), would not expire until July 23, 2015.

The requirements of 35 U.S.C. § 156(a) and 156(c)(4) have been satisfied as follows:

a) U.S. Patent No. 6,020,329 claims the active ingredient of CONVENIA® (cefovecin sodium).

b) U.S. Patent No. 6,020,329 is currently set to expire on July 22, 2011 (i.e., the term of the patent has not yet expired).

c) The term of U.S. Patent No. 6,020,329 has never been extended under subsection (e)(1) of 35 U.S.C. § 156.

d) This application for extension is being submitted by Pfizer Inc., the assignee of record of U.S. Patent No. 6,020,329, by its agent, in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. § 156(d).

e) The product CONVENIA® (cefovecin sodium), has been subject to a regulatory review period under section 512 of the Federal Food, Drug and Cosmetic Act before its commercial marketing or use, and permission for said commercial marketing or use is the first permitted commercial marketing or use under section 512 of the Federal Food, Drug and Cosmetic Act.

f) No patent that claims CONVENIA® (cefovecin sodium) has to this date been extended, nor has any other extension been applied for, under subsection (e)(1) of 35 U.S.C. § 156, for the regulatory review period which forms the basis for this application for the extension of the term of U.S. Patent No. 6,020,329.

The length of extension of the term of U.S. Patent No. 6,020,329 of 1462 days claimed by Applicant is determined according to the provisions of 37 CFR § 1.778 as follows:

a) According to 37 CFR § 1.778(b), the length of extension is equal to the regulatory review period for the approved product, reduced as appropriate according to paragraphs (d)(1) through (d)(6) 37 CFR § 1.778.

b) According to 37 CFR § 1.778(c), the regulatory review period is the sum of

(A) The number of days in the period beginning on the earlier of the date of a major health or environmental effects test on the drug was initiated or the date on which an exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective for the approved animal drug and ending on the date the NADA for the approved product was initially submitted under section 512 of the Federal Food, Drug and Cosmetic Act, and

(B) The number of days in the period beginning on the date the NADA was initially submitted under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act and ending on the date the NADA was approved.

The exemption under subsection (j) of section 512 became effective on November 16, 1999. NADA No. 141-285 was submitted to the FDA on March 15, 2008. As indicated in paragraph 11, NADA No. 141-285 was approved on April 25, 2008. This application for extension of the term of U.S. Patent 6,020,329 is based on the regulatory review period that ended with the approval of NADA No. 141-285 on April 25, 2008. Therefore, the length of the regulatory review period under 37

CFR § 1.778(c) is the sum of the period from November 16, 1999, to March 15, 2008, and from March 15, 2008, to April 25, 2008. This is the sum of 3043 days and 42 days, which is 3085 days.

c) According to 37 CFR § 1.778(d)(1)(i), the number of days in the regulatory review period which were on or before the date on which the patent issued must be subtracted from the number of days in the regulatory review period. U.S. Patent No. 6,020,329 issued on February 1, 2000. The number of days in the regulatory review period was 3085 days. Subtraction of the period on or before the patent issuance (77 days) from the regulatory review period (3085 days) leaves a reduced regulatory period of from February 1, 2000, to March 15, 2008, and from March 15, 2008, to April 25, 2008. This is the sum of 2966 days and 42 days, which is 3008 days.

d) Under 37 CFR § 1.778(d)(1)(iii), the regulatory review period must be reduced by one-half of the period determined under 37 CFR § 1.778(c)(1) after that period is reduced in accordance with paragraph (d)(1)(i). As indicated above in paragraph 13 of this application, the period determined under CFR § 1.778(c)(1) is 3085 days. Subtracting the 77 days in the period on or before the patent issuance reduces the period in (c)(1) to 3008 days. One-half of 3008 days is 1504 days. Subtracting this amount (1504 days), ignoring half days in the subtraction, from 2966 days (the portion of the regulatory review period that occurred after issuance of the patent) leaves a reduced regulatory review period of 1462 days. (37 CFR § 1.778(d)(1)(iii)).

e) According to 37 CFR § 1.778(d)(2), the reduced regulatory review period of 1462 days is added to the expiration date of U.S. Patent No. 6,020,329 (July 22, 2011) to give an extended expiration date of July 23, 2015 (37 CFR § 1.778(d)(2)).

f) According to 37 CFR § 1.778(d)(3)), when 14 years is added to the date of approval of the NADA application under section 512 of the Federal

Food, Drug and Cosmetic Act, (April 25, 2008), this gives a date of April 25, 2022 (37 CFR § 1.778(d)(3)).

g) According to 37 CFR § 1.778(d)(4), by comparing the dates for the ends of the periods obtained pursuant to paragraphs (d)(2) and (d)(3) and selecting the earlier date, the date for extended expiration of U.S. Patent No. 6,020,329 is July 23, 2015.

h) The five-year limitation of 35 U.S.C. §156(g)(6)(A) and 37 CFR § 1.778(d)(5) applies to this application, because U.S. Patent No. 6,020,329 issued after the enactment of the Generic Animal Drug and Patent Term Restoration Act (November 16, 1988). When 5 years is added to the expiration of U.S. Patent No. 6,020,329 (July 22, 2011), this gives a date of July 22, 2016. This date is later than the date obtained according to 37 CFR § 1.778(d)(4), therefore, under 37 CFR § 1.778(d)(5), Applicant is entitled to an extension corresponding to the period of from July 22, 2011, to July 23, 2015. This is 1462 days, which is the length of extension being claimed. Hence, Applicant is in compliance with 35 U.S.C. §156(g)(6)(A) and 37 CFR § 1.778(d)(5).

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patent and Trademarks and the Secretary of Health and Human Services any information, which is material to the determination of entitlement to the 1462 day extension being sought to the term of the United States Patent No. 6,020,329.

(14) The prescribed fee for receiving and acting on this application for extension is to be charged to Deposit Account 16-1445, as authorized in the enclosed transmittal letter.

(15) Please address all inquiries and correspondence relating to this application for patent term extension to:

John H. Engelmann
Pfizer Inc.
7000 Portage Road (KZO-267-635)
Kalamazoo, Michigan 49001

(16) Pursuant to 37 C.F.R. § 1.740(b), two duplicates of these application papers, certified as such, are enclosed herewith.

(17) A declaration pursuant to 37 C.F.R. § 1.740(a)(17) and 1.740(b) is enclosed.

Respectfully submitted,

Dated: JUNE 20, 2008

John H. Engelmann
Attorney for Applicant
Registration No. 28,075

Pfizer Inc.
Patent Department
7000 Portage Road (KZO-267-635)
Kalamazoo, Michigan 49001
(269) 833-2532

Customer No. 25533

US Patent No. 6,020,329
Attorney Docket No. PC9137C

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE: U. S. PATENT NO. 6,020,329

ISSUED: FEBRUARY 1, 2000

TO: JOHN HARGREAVES BATESON, ET AL.

FOR: CEPHALOSPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

ASSIGNEE: PFIZER INC.

Declaration Accompanying Application for Extension of Patent Term Under 35 U.S.C. § 156

Mail Stop Hatch-Waxman PTE
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

I, John H. Engelmann, declare as follows:

1. I am a patent attorney. I am a member of the Bar of the State of Connecticut and I am authorized to practice before the Patent and Trademark Office, Registration No. 28,075.
2. I am employed by Pfizer Inc., a corporation of Delaware, having a place of business at 7000 Portage Road, Kalamazoo, Michigan 49001, which is wholly owned by Pfizer Inc., a corporation of Delaware, having a place of business at 235 East 42nd Street, New York, NY 10017. Pfizer Inc. is the owner of record of United States Patent No. 6,020,329.
3. I have general authority from Pfizer Inc. to act on its behalf in patent matters.
4. I have reviewed and I understand the contents of the application of Pfizer Inc., dated June 20, 2008, which is being submitted herewith for

extension of the term of United States Patent No. 6,020,329 under 35 U.S.C. § 156 and 37 C.F.R. § 1.730.

5. I believe that United States Patent No. 6,020,329 is subject to extension pursuant to 37 C.F.R. § 1.710.

6. I believe that the length of extension of term of United States Patent No. 6,020,329 which is being claimed by Pfizer Inc. is justified under 35 U.S.C. § 156.

7. I believe that the patent for which extension is being sought meets the conditions for extension of the term of the patent as set forth in 37 C.F.R. § 1.720.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application being submitted herewith or any extension of patent term granted thereon.

Signed this 20TH day of JUNE 2008, at Kalamazoo, Michigan.

John H. Engelmann

John H. Engelmann
Attorney for Applicant
Registration No. 28,075

Pfizer Inc.
Patent Department
7000 Portage Road (KZO-267-635)
Kalamazoo, Michigan 49001
(269) 833-2532

Customer No. 25533

US Patent No. 6,020,329
Attorney Docket No. PC9137C

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

RE: U. S. PATENT NO. 6,020,329

ISSUED: FEBRUARY 1, 2000

TO: JOHN HARGREAVES BATESON, ET AL.

FOR: CEPHALOSPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

ASSIGNEE: PFIZER INC.

Certification

Mail Stop Hatch-Waxman PTE
Office of Patent Legal Administration
Room MDW 7D55
600 Dulany Street (Madison Building)
Alexandria, VA 22314

Dear Sir:

I hereby certify that attached hereto is a duplicate copy of the application papers of Pfizer Inc., dated June 20, 2008, for extension of the term of United States Patent No. 6,020,329 under 35 U.S.C. § 156.

Dated: JUNE 20, 2008

John H. Engelmann
Attorney for Applicant
Registration No. 28,075

Pfizer Inc.
Patent Department
7000 Portage Road (KZO-267-635)
Kalamazoo, Michigan 49001
(269) 833-2532

EXHIBIT A

US006020329A

United States Patent [19]

Bateson et al.

[11] **Patent Number: 6,020,329**

[45] **Date of Patent: Feb. 1, 2000**

[54] **CEPHALOPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS**

[75] Inventors: **John Hargreaves Bateson; George Burton; Stephen Christopher Martin Fell**, all of Betchworth, United Kingdom

[73] Assignee: **Pzifer Inc.**, New York, N.Y.

[21] Appl. No.: **08/958,864**

[22] Filed: **Oct. 20, 1997**

Related U.S. Application Data

[63] Continuation of application No. 08/470,786, Jun. 6, 1995, abandoned, which is a continuation of application No. 07/934,667, Jan. 22, 1993, abandoned.

[30] **Foreign Application Priority Data**

Jul. 24, 1990 [GB] United Kingdom 9016189.4
May 2, 1991 [GB] United Kingdom 9109540.6

[51] **Int. Cl.**[7] **A61K 31/545**; A61K 31/435; C07D 501/20; C07D 463/00

[52] **U.S. Cl.** **514/202**; 540/222; 540/300; 540/301

[58] **Field of Search** 540/222, 300, 540/301; 514/202

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,939,157 2/1976 Nayler et al. ..... 260/243 C
3,959,267 5/1976 Nayler et al. ..... 260/243 C
3,962,223 6/1976 Martel et al. ..... 260/243 C
3,975,383 8/1976 Nayler et al. ..... 260/243 C
5,246,926 9/1993 Bateson et al. 514/202

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1385831 3/1975 United Kingdom .

OTHER PUBLICATIONS

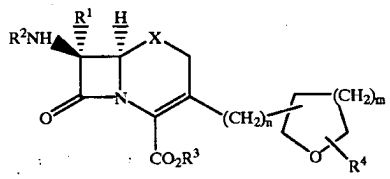
"Drugs of the Future", vol. 14, No. 1,1989, pp. 73–74.
"Drugs of the Future", vol. 13, No. 1, 1988, pp. 16–19.

Primary Examiner—Richard L. Raymond
Assistant Examiner—Pavanaram K Sripada
Attorney, Agent, or Firm—P. C. Richardson; P. H. Ginsburg; L. B. Ling

[57] **ABSTRACT**

β-Lactam antibiotics of formula (I) or a salt thereof, wherein R^1 is hydrogen, methoxy or formamido; R^2 is an acy group; CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group; R^4 represents up to four substituents; X is S, SO, SO_2, O or CH_2; m is 1 or 2; and n is 0, useful in the treatment of bacterial infec

(I)



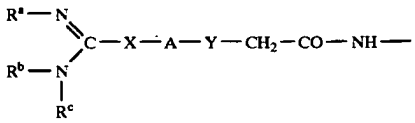
12 Claims, No Drawings

CEPHALOPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

This is a continuation of application Ser. No. 08/470,786, filed on Jun. 6, 1995, abandoned which is a continuation of application Ser. No. 07/934,667, filed Jan. 22, 1993 abandoned.

This invention relates to novel β-lactam containing compounds, their preparation and their use, and in particular to a novel class of cephalosporins. These compounds have antibacterial properties, and are therefore of use in the treatment of bacterial infections in humans and animals caused by a wide range of organisms.

GB 1 385 831 (Hoechst) claims 7-acylamino-cephemcarboxylic acid compounds substituted at the 7-position by a group:

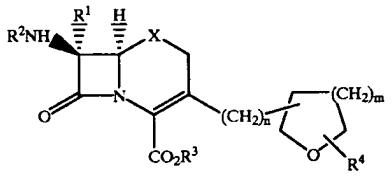


in which R^a and R^b, which may be the same or different, each represents a hydrogen atom or an alkyl group having from 1 to 5 carbon atoms or R^a and R^b together represent an alkylene group which may be substituted, R^c represents a hydrogen atom or an alkyl group having from 1 to 5 carbon atoms, X represents a single bond or an NH group, A represents a phenylene or thienylene group which may be substituted and Y represents a single bond or an oxygen atom;
and substituted at the 3-position by an alkyl group having from 1 to 5 carbon atoms, or a cyclo-alkyl group having from 3 to 7 ring carbon atoms which may include one or more hetero ring atoms. Tetrahydrofuranyl is described as an example of a 3-position substituent from a list of 14 radicals. The Examples describe only methyl, ethyl and isopropyl groups at the 3-position of the cephalosporin nucleus.

We have now found a particular class of cephalosporins bearing a cyclic ether substituent at the 3-position of the cephalosporin nucleus that possesses prolonged and high levels of antibacterial activity, and shows good absorption both parentally and orally, especially orally.

The present invention provides a compound of formula (I) or a salt thereof:

(I)



wherein

R^1 is hydrogen, methoxy or formamido;

R^2 is an acyl group, in particular that of an antibacterially active cephalosporin;

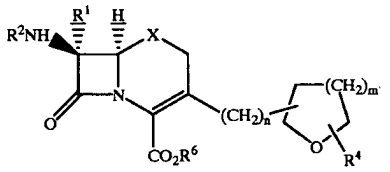
CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group (such as a pharmaceutically acceptable in vivo hydrolysable ester group); R^4 represents up to four substituents selected from alkyl, alkenyl, alkynyl, alkoxy, hydroxy, halogen, amino, alkylamino, acylamino, dialkylamino, CO_2R, $CONR_2$, SO_2NR_2 (where R is hydrogen or C_{1-6} alkyl), aryl and heterocyclyl, which may be the same or different and wherein any R^4 alkyl substituent is optionally substituted by any other R^4 substituent; X is S,SO,SO_2,O or CH_2; m is 1 or 2; and n is 0.

The bonding carbon atom of the cyclic ether moiety which links the ring to the cephalosporin nucleus is generally asymmetric. The present invention includes either stereoisomer, as well as mixtures of both isomers.

In compounds of formula (I) wherein R^1 is formamido, the formamido group can exist in conformations wherein the hydrogen atoms of the —NH—CHO moiety are cis- or trans-; of these the cis conformation normally predominates.

Since the β-lactam antibiotic compounds of the present invention are intended for use as therapeutic agents in pharmaceutical compositions, it will be readily appreciated that preferred compounds within formula (I) are pharmaceutically acceptable, i.e. are compounds of formula (Ia) or pharmaceutically acceptable salts or pharmaceutically acceptable in vivo hydrolysable esters thereof:

(Ia)



wherein R^1, R^2, R^4, m, n and X are as defined with respect to formula (I) and the group CO_2R^6 is CO_2R^3 where CO_2R^3 is a carboxy group or a carboxylate anion.

Accordingly, the present invention provides a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for use as a therapeutic agent, and in particular an in vivo hydrolysable ester thereof for use as an orally administrable therapeutic agent.

The present invention further provides a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for use in the treatment of bacterial infections, more particularly an in vivo—hydrolysable ester thereof for use in the oral treatment of bacterial infections.

The present invention also includes a method of treating bacterial infections in humans and animals which comprises the administration of a therapeutically effective amount of an antibiotic compound of this invention of the formula (Ia) or a pharmaceutically acceptable in vivo hydrolysable ester thereof, in particular the oral administration of a therapeutically effective amount of an in vivo hydrolysable ester.

In addition, the present invention includes the use of a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for the manufacture of a medicament for the treatment of bacterial infections, in particular the use of an in vivo hydrolysable ester for the manufacture of a medicament for the oral treatment of bacterial infections.

Those compounds of the formula (I) wherein R^3 is a readily removable carboxy protecting group other than a pharmaceutically acceptable in vivo hydrolysable ester or which are in non-pharmaceutically acceptable salt form are primarily useful as intermediates in the preparation of compounds of the formula (Ia) or a pharmaceutically acceptable salt or pharmaceutically acceptable in vivo hydrolysable ester thereof.

Suitable readily removable carboxy protecting groups for the group R^3 include groups forming ester derivatives of the carboxylic acid, including in vivo hydrolysable esters. The derivative is preferably one which may readily be cleaved in vivo.

It will be appreciated that also included within the scope of the invention are salts and carboxy-protected derivatives, including in vivo hydrolysable esters, of any carboxy groups that may be present as optional substituents in compounds of formula (I) or (Ia). Also included within the scope of the invention are acid addition salts of any amino group or substituted amino group that may be present as optional substituents in compounds of formula (I) or (Ia).

Suitable ester-forming carboxyl-protecting groups are those which may be removed under conventional conditions. Such groups for R^3 include benzyl, p-methoxybenzyl, benzoylmethyl, p-nitrobenzyl, 4-pyridylmethyl, 2,2,2-trichloroethyl, 2,2,2-tribromoethyl, t-butyl, t-amyl, allyl, diphenylmethyl, triphenylmethyl, adamantyl, 2-benzyloxyphenyl, 4-methylthiophenyl, tetrahydrofur-2-yl, tetrahydropyran-2-yl, pentachlorophenyl, acetonyl, p-toluenesulphonylethyl, methoxymethyl, a silyl, stannyl or phosphorus-containing group, an oxime radical of formula $-N=CHR^7$ where R^7 is aryl or heterocyclic, or an in vivo hydrolysable ester radical such as defined below.

When used herein the term 'aryl' includes phenyl and naphthyl, each optionally substituted with up to five, preferably up to three, groups selected from halogen, mercapto, C_{1-6} alkyl, phenyl, C_{1-6} alkoxy, hydroxy(C_{1-6})alkyl, mercapto(C_{1-6})alkyl, halo(C_{1-6}) alkyl, hydroxy, amino, nitro, carboxy, C_{1-6} alkylcarbonyloxy, alkoxycarbonyl, formyl, or C_{1-6} alkylcarbonyl groups.

The terms 'heterocyclyl' and 'heterocyclic' as used herein include aromatic and non-aromatic, single and fused, rings suitably containing up to four hetero-atoms in each ring selected from oxygen, nitrogen and sulphur, which rings may be unsubstituted or substituted by, for example, up to three groups selected from halogen, (C_{1-6})alkyl, (C_{1-6}) alkoxy, halo(C_{1-6})alkyl, hydroxy, carboxy, carboxy salts, carboxy esters such as (C_{1-6})alkoxycarbonyl, (C_{1-6}) alkoxycarbonyl(C_{1-6})alkyl, aryl, and oxo groups. Each heterocyclic ring suitably has from 4 to 7, preferably 5 or 6, ring atoms. The term 'heteroaryl' refers to heteroaromatic heterocyclic rings. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring. Compounds within the invention containing a heterocyclyl group may occur in two or more tautometric forms depending on the nature of the heterocyclyl group; all such tautomeric forms are included within the scope of the invention.

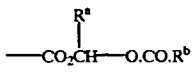
When used herein the terms 'alkyl' alkenyl, alkynyl and 'alkoxy' include straight and branched chain groups containing from 1 to 6 carbon atoms, such as methyl, ethyl, propyl and butyl. A particular alkyl group is methyl.

When used herein the term 'halogen' refers to fluorine, chlorine, bromine and iodine.

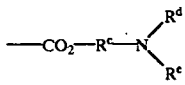
A carboxyl group may be regenerated from any of the above esters by usual methods appropriat to the particular R^3 group, for example, acid- and base-catalysed hydrolysis, or by enzymically-catalysed hydrolysis, or by hydrogenolysis under conditions wherein the remainder of the molecule is substantially unaffected.

Examples of suitable pharmaceutically acceptable in vivo hydrolysable ester groups include those which break down readily in the human body to leave the parent acid or its salt. Suitable ester groups of this type include those of part formulae (i), (ii), (iii), (iv) and (v):

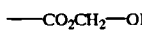
(i)



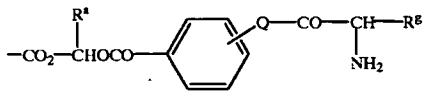
(ii)



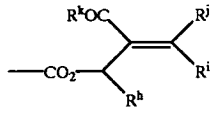
(iii)



(iv)



(v)

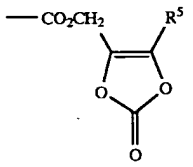


wherein R^a is hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, methyl, or phenyl, R^b is C_{1-6} alkyl, C_{1-6} alkoxy, phenyl, benzyl, C_{3-7} cycloalkyl, C_{3-7} cycloalkyloxy, C_{1-6} alkyl C_{3-7} cycloalkyl, 1-amino C_{1-6} alkyl, or 1-(C_{1-6} alkyl)amino C_{1-6} alkyl; or R^a and R^b together form a 1,2-phenylene group optionally substituted by one or two methoxy groups; R^c represents C_{1-6} alkylene optionally substituted with a methyl or ethyl group and R^d and R^e independently represent C_{1-6} alkyl; R^f represents C_{1-6} alkyl; R^g represents hydrogen or phenyl optionally substituted by up to three groups selected from halogen, C_{1-6} alkyl, or C_{1-6} alkoxy; Q is oxygen or NH; R^h is hydrogen or C_{1-6} alkyl; R^i is hydrogen, C_{1-6} alkyl optionally substituted by halogen, C_{2-6} alkenyl, C_{1-6} alkoxycarbonyl, aryl or heteroaryl; or R^h and R^i together form C_{1-6} alkylene; R^j represents hydrogen, C_{1-6} alkyl or C_{1-6} alkoxycarbonyl; and R^k represents C_{1-8} alkyl, C_{1-8} alkoxy, C_{1-6} alkoxy(C_{1-6})alkoxy or aryl.

Examples of suitable in vivo hydrolysable ester groups include, for example, acyloxyalkyl groups such as acetoxymethyl, pivaloyloxymethyl, α-acetoxyethyl, α-pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, and (1-aminoethyl)carbonyloxymethyl; alkoxycarbonyloxyalkyl groups, such as ethoxycarbonyloxymethyl, α-ethoxycarbonyloxyethyl and propoxycarbonyloxyethyl; dialkylaminoalkyl especially di-loweralkylamino alkyl groups such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl or diethylaminoethyl; 2-(alkoxycarbonyl)-2-alkenyl groups such as 2-(isobutoxycarbonyl)pent-2-enyl and 2-(ethoxycarbonyl) but-2-enyl; lactone groups such as phthalidyl and dimethoxyphthalidyl; and esters linked to a second β-lactam antibiotic or to a β-lactamase inhibitor.

A preferred in vivo hydrolysable ester group is the pivaloyloxymethyl ester.

A further suitable pharmaceutically acceptable in vivo hydrolysable ester group is that of the formula:



wherein R^5 is hydrogen, C_{1-6} alkyl or phenyl.

Suitable pharmaceutically acceptable salts of the carboxy group of the compound of formula (I) include metal salts, eg aluminium, alkali metal salts such as sodium or potassium, especially sodium, alkaline earth metal salts such as calcium or magnesium, and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy-lower alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine or tris-(2-hydroxyethyl)- amine, cycloalkylamines such as dicyclohexylamine, or with procaine, dibenzylamine, N,N-dibenzylethylene- diamine, 1-ephenamine, N-methylmorpholine, N-ethylpiperidine, N-benzyl-β-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, ethylenediamine, or bases of the pyridine type such as pyridine, collidine or quinoline, or other amines which have been used to form salts with known penicillins and cephalosporins. Other useful salts include the lithium salt and silver salt. Salts within compounds of formula (I), may be prepared by salt exchange in conventional manner.

In compounds of formula (I) or (Ia), the group X may be sulphur or an oxidised sulphur atom, i.e. a sulphoxide (SO) or sulphone (SO_2) group. When X is a sulphoxide group it will be understood that α- and β-isomers may exist; both such isomers are encompassed within the scope of the present invention.

Examples of X include S, SO, SO_2 and CH_2. Preferably X is sulphur or CH_2.

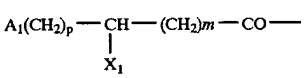
Advantageously, R^1 is hydrogen.

Suitably, the cyclic ether at the 3-position of the cephalosporin nucleus is unsubstituted or substituted by up to three substituents, R^4, selected from C_{1-6} alkyl, for example methyl, C_{1-6} alkoxy, for example methoxy, C_{1-6} alkoxycarbonyl for example methoxycarbonyl, C_{1-6} alkoxy C_{1-6} alkyl, for example methoxymethyl, and C_{1-6} alkanoyloxy C_{1-6} alkyl, for example acetoxymethyl. Preferably the cyclic ether at the 3-position of the cephalosporin nucleus is unsubstituted.

Preferably m is 1.

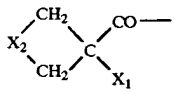
Preferably the cyclic ether is bonded to the cephalosporin nucleus at a ring carbon adjacent to the oxygen heteroatom.

Suitable acyl groups R^2 include those of formulae (a)–(f):

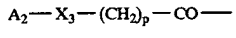


(a)

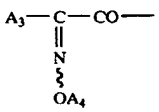
(b)



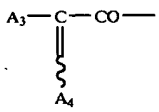
(c)



(d)



(e)



(f)

wherein p is 0, 1 or 2; m is 0, 1 or 2; A_1 is C_{1-6} alkyl, substituted C_{1-6} alkyl, C_{3-6} cycloalkyl, cyclohexenyl, cyclohexadienyl, an aromatic (including heteroaromatic) group, such as phenyl, substituted phenyl, thienyl, pyridyl, or an optionally substituted thiazolyl group, a C_{1-6} akylthio group or C_{1-6} alkyloxy; X_1 is a hydrogen or halogen atom, a carboxylic acid, carboxylic ester, sulphonic acid, azido, tetrazolyl, hydroxy, acyloxy, amino, ureido, acylamino, heterocyclylamino, guanidino or acylureido group; A_2 is an aromatic group, for example a phenyl, 2,6-dimethoxyphenyl,2-alkoxy-1-naphthyl, 3-arylisoxazolyl, or a 3-aryl-5-methylisoxazolyl group, such as 3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl; a substituted alkyl group; or a substituted dithietane; X_2 is a $—CH_2OCH_2—$, $—CH_2SCH_2—$ or alkylene group; X_3 is an oxygen or sulphur atom; A_3 is an aryl or heteroaryl group such as phenyl, substituted phenyl, furyl, aminothiazolyl or aminothiadiazolyl in which the amino group is optionally protected; and A_4 is hydrogen, C_{1-6}alkyl, C_{3-8} cycloalkyl, C_{3-8} cycloalkyl(C_{1-6})alkyl, C_{1-6} alkoxycarbonyl(C_{1-6}) alkyl, C_{2-6} alkenyl, carboxy(C_{1-6})alkyl, C_{2-6} alkynyl, aryl or C_{1-6}alkyl substituted by up to three aryl groups.

The term 'heteroaryl' as used herein means a heteroaromatic heterocyclic ring or ring system, suitably having 5 or 6 ring atoms in each ring.

Suitably when R^2 is a group (a), A_1 is C_{1-6} alkyl, C_{3-6} cycloalkyl, cyclohexenyl, cyclohexadienyl, phenyl, substituted phenyl such as hydroxyphenyl, thienyl or pyridyl; and X_1 is a hydrogen or halogen atom, or a carboxy, carboxylic ester, azido, tetrazolyl, hydroxy, acyloxy, optionally protected amino, ureido, guanidino or acylureido group.

Suitably when R^2 is a group of formula (d), A_2 is phenyl, X_3 is oxygen and p is 0.

Alternatively when R^2 is a group of formula (e) or (f) suitable values for the group A_3 include those commonly found in antibacterially active cephalosporins containing a hydroxyimino, substituted hydroxyimino or vinyl group in the side chain attached to position 7 of the cephalosporin nucleus, for example phenyl, thien-2-yl, thien-3-yl, fur-2-yl, fur-3-yl, pyrid-2-yl, pyrid-3-yl, pyrid-4-yl, 5-amino-1,2,4-thiadiazol-3-yl and 2-aminothiazol-4-yl in each of which the amino group is optionally protected.

Preferred groups for A_3 include phenyl, 2-aminothiazol-4-yl, fur-2-yl, thien-2-yl, 2-(2-chloroacetamido)thiazol-4-yl, 2-tritylamino-thiazol-4-yl, 5-amino-1,2,4-thiadiazol-3-yl and 4-aminopyrimid-2-yl.

In compounds of formula (Ia), a particularly preferred group for A_3 is 2-aminothiazol-4-yl.

Suitable values for the group A_4 include hydrogen, methyl, ethyl, cyclopropylmethyl, triphenylmethyl (trityl), cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, phenyl, carboxymethyl, carboxypropyl and t-butoxycarbonylmethyl.

Preferred values for A_4 in compounds of formula (Ia) include methyl and hydrogen.

It will be appreciated that compounds of the invention wherein R^2 is a group of formula (e) (or (f)) can exist as syn and anti (or E and Z) isomers or mixtures thereof. Both isomers are encompassed within the scope of this invention.

Preferably the compounds of the invention wherein R^2 is a group of formula (e) have the syn configuration (i.e. have the group OA_4 syn to the amide linkage) or are enriched in that isomer.

Similarly, when R^2 is a group of formula (f) , the group A_4 is preferably cis to the amide linkage, i.e. when group (f) is 2-amino-thiazol-4-yl, the Z-configuration is preferred.

Certain compounds of the invention include an amino group which may be protected. Suitable amino protecting groups are those well known in the art which may be removed under conventional conditions without disruption of the remainder of the molecule.

Examples of amino protecting groups include C_{1-6} alkanoyl; benzoyl; benzyl optionally substituted in the phenyl ring by one or two substituents selected from C_{1-4} alkyl, C_{1-4} alkoxy, trifluoromethyl, halogen, or nitro; C_{1-4} alkoxycarbonyl; benzyloxycarbonyl or trityl substituted as for benzyl above; allyloxycarbonyl,trichloroethoxycarbonyl or chloroacetyl.

Some of the compounds of this invention may be crystallised or recrystallised from solvents such as organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Since the antibiotic compounds of the invention are intended for use in pharmaceutical compositions it will readily be understood that they are each provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 95% pure (% are on a weight for weight basis). Impure preparations of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions; these less pure preparations of the compounds should contain at least 1%, more suitably at least 5% and preferably from 10 to 49% of a compound of the formula (I) or salt thereof.

Specific compounds within this invention of formula (Ia) include the following pharmaceutically acceptable carboxylic acids, salts and in-vivo hydrolysable esters:

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylate;

pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7[-2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl] ceph-3-em-4-carboxylate;

pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate;

(6R, 7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylic acid;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-3-yl] ceph-3-em-4-carboxylate;

acetoxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5-methoxymethyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-pent-2-enamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiadiazol-4-yl)-^{2}-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate;

(RS)-1-acetoxyethyl (6R,7R)-7-[2-(2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

(6R, 7R) -7- [2- (2-aminothiazol-4-yl)-2-(Z)-carboxymethoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylic acid, disodium salt;

sodium (6R,7R)-7-[(R)-2-amino-2-(4-hydroxyphenyl) acetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (1S,6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S) -tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate-1-oxide;

sodium 7-[2- (2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(tetrahydrofuran-2-yl)-1-carba-1-dethiaceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate-1,1-dioxide;

(RS)-1-(propan-2-yl)oxycarbonyloxyethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,5R)-5-methyltetrahydrofuran-2-yl)-ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(furan-2-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]-ceph-3-em-4-carboxylate;

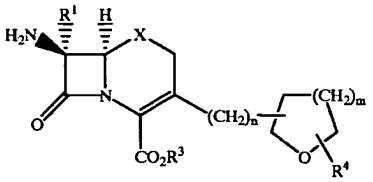
sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5-methoxycarbonyltetrahydrofuran-2-yl) -ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[-methyltetrahydrofuran-2-yl] ceph-3-em-4-carboxylate; and

2-ethoxycarbonyl-(Z)-but-2-enyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetomido]-3-(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

The present invention further provides a process for the preparation of a compound of formula (I), which process comprises treating a compound of formula (II) or a salt thereof:

(II)



wherein R^1, CO_2R^3, R^4, m, n and X are as hereinbefore defined, wherein any reactive groups may be protected, and wherein the amino group is optionally substituted with a group which permits acylation to take place; with an N-acylating derivative of an acid of formula (III):

$$R^2OH \quad \text{(III)}$$

wherein R^2 is as defined with respect to formula (I) and wherein any reactive groups may be protected; and thereafter, if necessary or desired, carrying out one or more of the following steps:

i) removing any protecting groups;

ii) converting the group CO_2R^3 into a different group CO_2R^3;

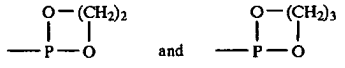
iii) converting the group R^2 into a different group R^2;

iv) converting the group X into a different group X;

v) converting the product into a salt.

Acids of formula (III) may be prepared by methods known in the art, or methods analogous to such processes. Suitable processes include those described, for example, in UK Patent 2 107 307 B, UK Patent Specification No. 1,536,281, and U.K. Patent Specification No. 1,508,064.

Suitable groups which permit acylation to take place and which are optionally present on the amino group of the starting material of the formula (II) include N-silyl, N-stannyl and N-phosphorus groups, for example trialkylsilyl groups such as trimethylsilyl, trialkyltin groups such as tri-n-butyltin, groups of formula $—P.R^{20}R^{21}$ wherein R^{20} is an alkyl, haloalkyl, aryl, aralkyl, alkoxy, haloalkyl, aryl, aralkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy or dialkylamino group, R^{21} is the same as R^{20} or is halogen or R^{20} and R^{21} together form a ring; suitable such phosphorus groups being $—P(OC_2H_5)_2$, $—P(C_2H_5)_2$,



A group which may optionally be introduced onto the amino group in the compound of formula (II) is trimethylsilyl.

Advantageously the silylation reaction may be carried out in situ, prior to the acylation reaction, with a silylating agent that does not require concomitant addition of base. Suitable silylating agents include, for example, N-(trimethylsilyl)-acetamide, N,O-bis- (trimethylsilyl) acetamide, N,O-bis (trimethylsilyl)-trifluoroacetamide, N-methyl-N-trimethylsilylacetamide, N-methyl-N-trimethylsilyl-trifluoroacetamide, N,N'-bis(trimethylsilyl)urea, and N,O-bis(trimethylsilyl)carbamate. A preferred silylating agent is N,O-bis(trimethylsilyl)acetamide. The silylation reaction may suitably be carried out in an inert, anhydrous organic solvent such as dichloromethane at room temperature or at an elevated temperature, for example 30–60° C., preferably 40–50° C.

The above process may optionally be carried out in the presence of a small quantity, for example 0.1 equivalents, of a silyl halide, for example a tri(C_{1-6})alkylsilyl halide, especially trimethylsilyl chloride.

A reactive N-acylating derivative of the acid (III) is employed in the above process. The choice of reactive derivative will of course be influenced by the chemical nature of the substituents of the acid.

Suitable N-acylating derivatives include an acid halide, preferably the acid chloride or bromide or alternatively a symmetrical or mixed anhydride. The acylation may be effected in the presence of an acid binding agent for example, tertiary amine (such as pyridine or dimethylaniline), molecular sieves, an inorganic base (such as calcium carbonate or sodium bicarbonate) or an oxirane, which binds hydrogen halide liberated in the acylation reaction. The oxirane is preferably a (C_{1-6})-1,2-alkylene oxide—such as ethylene oxide or propylene oxide. The acylation reaction using an acid halide may be carried out at a temperature in the range −50° C. to +50° C., preferably −20° C. to +20° C., in aqueous or non-aqueous media such as water, acetone, tetrahydrofuran, ethyl acetate, dimethylacetamide, dimethylformamide, acetonitrile, dichloromethane, 1,2-dichloroethane, or mixtures thereof. Alternatively, the reaction may be carried out in an unstable emulsion of water-immiscible solvent, especially an aliphatic ester or ketone, such as methyl isobutyl ketone or butyl acetate. The acylation with acid halide or anhydride is suitably carried out in the presence of a basic catalyst such as pyridine or 2,6-lutidine.

Acid halides may be prepared by reacting the acid (III) or a salt or a reactive derivative thereof with a halogenating (eg chlorinating or brominating) agent such as phosphorus pentachloride, thionyl chloride, oxalyl chloride or phosgene.

Suitable mixed anhydrides are anhydrides with, for example, carbonic acid monoesters, trimethyl acetic acid, thioacetic acid, diphenylacetic acid, benzoic acid, phosphorus acids (such as phosphoric, phosphorous, and phosphinic acids) or aromatic or aliphatic sulphonic acids (such as p-toluenesulphonic acid or methanesulphonic acid).

Alternative N-acylating derivatives of acid (III) are the acid azide, or activated esters such as esters with 2-mercaptopyridine, cyanomethanol, p-nitrophenol, 2,4-dinitrophenol, thiophenol, halophenols, including pentachlorophenol, monomethoxyphenol, N-hydroxy succinimide, N-hydroxybenzotriazole, or 8-hydroxyquinoline; or amides such as N-acylsaccharins, N-acylthiazolidin-2-thione or N-acylphthalimides; or an alkylidene iminoester prepared by reaction of the acid (III) with an oxime.

Other reactive N-acylating derivatives of the acid (III) include the reactive intermediates formed by reaction in situ with a condensing agent such as a carbodiimide, for example, N,N'-diethyl-, dipropyl- or diisopropylcarbodiimide, N,N'-di-cyclohexyl-carbodiimide, or N-ethyl-N'-[3-(dimethylamino)propyl]-carbodiimide; a suitable carbonyl compound, for example, N,N'-carbonyldiimidazole or N,N'-carbonyldi-triazole; an isoxazolinium salt, for example, N-ethyl-5-phenylisoxazolinium-3-sulphonate or N-t-butyl-5-methylisoxazolinium perchlorate; or an N-alkoxycarbonyl 2-alkoxy-1,2-dihydroquinoline, such as N-ethoxycarbonyl 2-ethoxy-1,2-dihydroquinoline. Other condensing agents include Lewis acids (for example BBr_3-C_6H_6); or a phosphoric acid condensing agent such as diethylphosphorylcyanide. The condensation reaction is preferably carried out in an organic reaction medium, for example, methylene

chloride, dimethylformamide, acetonitrile, alcohol, benzene, dioxan or tetrahydrofuran.

A further method of forming the N-acylating derivative of the acid of formula (III) is to treat the acid of formula (III) with a solution or suspension preformed by addition of a carbonyl halide, preferably oxalyl chloride, or a phosphoryl halide such as phosphorus oxychloride, to a halogenated hydrocarbon solvent, preferably dichloromethane, containing a lower acyl tertiary amide, preferably N,N-dimethylformamide. The N-acylating derivative of the acid of formula (III) so derived may then be caused to react with a compound of formula (II). The acylation reaction may conveniently be carried out at −40° to +30° C., if desired in the presence of an acid binding agent such as pyridine. A catalyst such as 4-dimethylaminopyridine may optionally also be added. A preferred solvent for the above acylation reaction is dichloromethane.

The optional reduction step, the optional conversion of R^2 to a different R^2, CO_2R^3 to a different CO_2R^3 and X to a different X, and the optional formation of a salt, may be carried out using methods well known in the art of cephalosporin and penicillin chemistry.

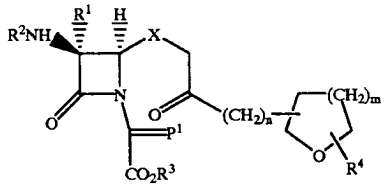
For example, when the group X is S, SO, or SO_2, the group X may be converted into a different group X by methods of oxidation or reduction well known in the art of cephalosporin and penicillin synthesis, as described, for example, in European Patent Application Publication No. 0 114 752. For example, sulphoxides (in which X is SO) may be prepared from the corresponding sulphide (in which X is S) by oxidation with a suitable oxidising agent, for example an organic peracid such as m-chloroperbenzoic acid.

A reduction step is generally effected by processes well known in the art of β-lactam chemistry, for example using phosphorus trichloride in dimethylformamide.

In the process described hereinabove, and in the process described hereinbelow, it may be necessary to remove protecting groups. Deprotection may be carried out by any convenient method known in the art such that unwanted side reactions are minimised. Separation of unwanted by-products may be carried out using standard methods.

In a further process of the invention, compounds of formula (I) may be prepared by cyclising a compound of formula (IV):

(IV)



wherein X, R^1, R^2, R^4, m, n and CO_2R^3 are as hereinbefore defined and P' is a phosphorus residue; and thereafter if necessary or desired, carrying out one or more of the following steps:

i) removing any protecting groups;

ii) converting the group CO_2R^3 into a different group CO_2R^3;

iii) converting the group R^2 into a different group R^2;

iv) converting the group X into a different group X;

v) converting the product into a salt.

The cyclisation reaction is an intramolecular Wittig-type reaction and is typically carried out by heating the compound of formula (IV) in an organic solvent system, for example in toluene, optionally in the presence of a suitable acid such as benzoic acid.

The phosphorus residue, P' is typically a trialkylphosphoranylidene residue, for example a C_{1-6} trialkylphosphoranylidene residue such as tri-n-butylphosphoranylidene, or a triarylphosphoranylidene residue such as triphenylphosphoranylidene.

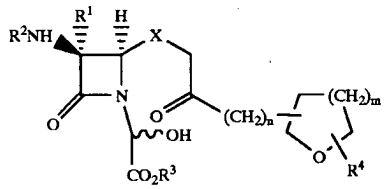
Where R^2 in a compound of formula (I) is required to be different from the group R^2 in the compound of formula (IV), the conversion may be effected via the intermediacy of a compound of formula (II) which has an amino group at the 7-position of the cephalosporin nucleus.

An R^2 side-chain may be removed by the Delft procedure commonly used in β-lactam chemistry. Suitable reaction conditions include treatment with phosphorus pentachloride and N-methylmorpholine at reduced temperature.

Compounds of formula (II) are novel compounds and as such form part of the invention.

A compound of formula (IV) may be prepared from a compound of formula (V):

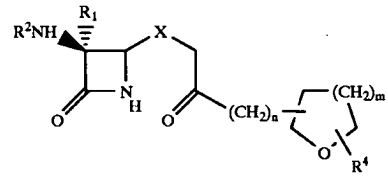
(V)



wherein X, R^1, R^2, R^4, m, n and CO_2R^3 are as hereinbefore defined, by reaction with a halogenating agent, suitably a chlorinating agent such as thionyl chloride, which reaction displaces the formula (V) hydroxyl group by halogen, suitably chloride, and is typically carried out at reduced temperature in an inert solvent, for example in tetrahydrofuran, in the presence of a base, typically a pyridine derivative such as 2,6-lutidine. Formation of the phosphorane may be effected by treatment of the halo-intermediate with an appropriate phosphine derivative, for example tri-n-butylphosphine or triphenylphosphine, suitably at ambient temperature in an inert solvent such as dioxan.

A compound of formula (V) may be prepared by reaction of a compound of formula (VI):

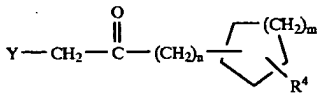
(VI)



wherein X, R^1, R^2, R^4, m and n are as hereinbefore defined with an ester of glyoxylic acid ($OCHCO_2R^3$) in the presence of triethylamine.

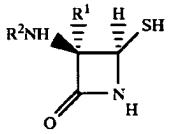
In a typical preparation of a compound of formula (VI) in which X is sulphur, a compound of formula (VII):

(VII)



wherein Y is a leaving group and R^4, m and n are as hereinbefore defined is reacted with a compound of formula (VIII):

(VIII)

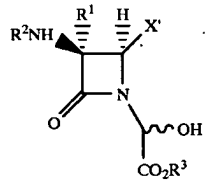


wherein R^1 and R^2 are as hereinbefore defined.

Suitably, a leaving group Y is halogen, for example chloro. The reaction may be carried out at ambient temperature in an inert solvent, for example acetone or dimethylformamide, in the presence for a base, for example potassium carbonate.

A compound of formula (V) may also be prepared by reaction of a compound of formula (IX):

(IX)



wherein R^1, R^2 and CO_2R^3 are as hereinbefore defined and X' is an X-group precursor, with a compound of formula (VII) as hereinbefore defined.

In a typical preparation of a compound of formula (V) in which X is sulphur, a Y leaving group in a compound of formula (VII), suitably a halogen such as chloro or bromo, is displaced by an X' mercapto group in a compound of formula (IX). The reaction may be carried out at ambient temperature in an inert solvent, for example acetone, with the addition of base, for example potassium carbonate, before work-up.

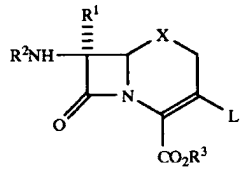
Azetidin-2-one compounds of formulae (VIII) and (IX) may be prepared according to known methods in heterocyclic synthetic chemistry and particularly by known methods in the art of β-lactam chemistry. For example a compound of formula (VIII) may be prepared according to the method of Osborne N. F. et al., J. Chem. Soc., Perkin Trans. I, 146, 1980.

A compound of formula (IX) in which X' is a mercapto group may be prepared by ring opening of a 4-thia-2,6-diazabicyclo [3.2.0]-hept-2-ene-7-one derivative according to the method of Masayuki Narisada et al., Tetrahedron Lett., 1755 (1978).

Compounds of formula (VII) are known compounds or may be prepared by standard methodology. For example, the compounds of formula (VII) in which Y is chloro or bromo may be prepared from the corresponding carboxylic acid (Y=COOH) via formation of the acid chloride followed by treatment with diazomethane and reaction of the resulting diazo compound with hydrogen chloride or hydrogen bromide.

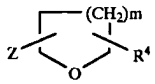
In a further process of the invention, compounds of formula (I) may be prepared directly by organo-cuprate displacement of a leaving group at the 3-position of a compound of formula (X):

(X)



wherein R^1, R^2, CO_2R^3 and X are as hereinbefore defined and L is a leaving group, suitably a mesylate, triflate or fluorosulphonate leaving group, by reaction with a compound of formula (XI):

(XI)

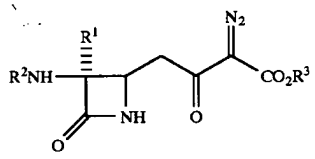


wherein Z is an organo-cuprate group and R^4 and m are as hereinbefore defined.

A compound with a 3-position leaving group, L, in which X is sulphur may be prepared by the procedure of Farina V. et al., J. Org. Chem., 54, 4962, (1989).

A compound with a 3-position leaving group, L, in which X is CH_2 may be prepared by a transition metal-catalysed carbenoid insertion reaction of a diazodicarbonyl compound of formula (XII):

(XII)



wherein R^1, R^2 and CO_2R^3 are as hereinbefore defined, followed by reaction with an appropriate anhydride, for example triflic anhydride. Compounds of formula (XII) may be prepared by the procedure of Bodurow C. and Carr M. A.; Tetrahedron Lett., 30 4801, (1989).

It should be noted that in processes of this invention Δ2-cephems may function as intermediates, in the synthetic sequences. Subsequent isomerisation steps by methods well known in cephalosporin chemistry will provide the Δ^3-cephems of the invention.

The present invention also provides a pharmaceutical composition which comprises a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof and a pharmaceutically acceptable carrier. The compositions of the invention include those in a form adapted for oral, topical or parenteral use and may be used for the treatment of bacterial infection in mammals including humans.

The antibiotic compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics.

The composition may be formulated for administration by any route, such as oral, topical or parenteral, especially oral. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit αose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol,-or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

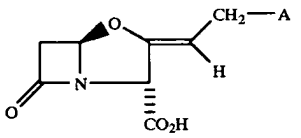
The compositions may contain from 0.1% by weight, preferably from 10–60% by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will preferably contain from 50–500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

No unacceptable toxicological effects are expected when a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof is administered in the above-mentioned dosage range.

The compound of formula (Ia) may be the sole therapeutic agent in the compositions of the invention or a combination with other antibiotics or with a β-lactamase inhibitor may be employed.

Advantageously, the compositions also comprise a compound of formula (XIII) or a pharmaceutically acceptable salt or ester thereof:

(XIII)

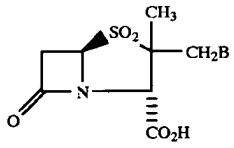


wherein

A is hydroxyl, substituted hydroxyl, thiol, substituted thiol, amino, mono- or di-hydrocarbyl-substituted amino, or mono- or di-acylamino; an optionally substituted triazolyl group; or an optionally substituted tetrazolyl group as described in EP-A-0 053 893.

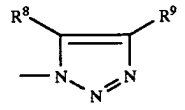
A further advantageous composition comprises a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof together with a compound of formula (XIV) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof:

(XIV)



wherein

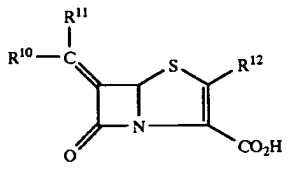
B represents hydrogen, halogen or a group of formula:



in which R^8 and R^9 are the same or different and each represents hydrogen, C_{1-6} alkoxycarbonyl or carboxy, or a pharmaceutically acceptable salt thereof.

Further suitable β-lactamase inhibitors include 6-alkylidene penems of formula (XV):

(XV)



or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, wherein R^{10} and R^{11} are the same or different and each represents hydrogen, or a C_{1-10}-hydrocarbon or heterocyclic group optionally substituted with a functional group; and R^{12} represents hydrogen or a group of formula R^{13} or —SR^{13} where R^{13} is an optionally substituted C_{1-10} hydrocarbon or heterocyclic group, as described in EP-A-0 041 768.

Further suitable β-lactamase inhibitors include 6β-bromopenicillanic acid and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof and 6β-iodopenicillanic acid and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof described in, for example, EP-A-0 410 768 and EP-A-0 154 132 (both Beecham Group).

Such compositions of this invention which include a β-lactamase inhibitory amount of a β-lactamase inhibitor are formulated in a conventional manner using techniques and procedures per se known in the art.

The antibiotic compounds of the present invention are active against a wide range of organisms including both Gram-negative organisms such as *E.coli* and Gram-positive organisms such as *S.aureus*.

The following Examples illustrate the preparation of compounds of the invention and intermediates thereto. The following biological data illustrate the activity of compounds of the invention in the form of MIC values (minimum inhibitory concentration) against a sample E.coli organism (NCTC 10418) and a sample *S.aureus* organism (*S.aureus* Oxford).

EXAMPLE 1

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) (RS)-2-Chloroacetyltetrahydrofuran

Oxalyl chloride (5.2ml, 60mmol) and DMF (1 drop) were added to (RS)-2-tetrahydrofuroic acid (W. E. Kaufmann and R. Adams, *J.Amer.Chem.Soc.*, 1923, 45, 3029) (4.64g, 40mmol) in dichloromethane (25ml). The mixture was stirred 1h, evaporated in vacuo, dichloromethane added and reevaporated to give 2-tetrahydrofuroyl chloride, ν_{max} (CH_2Cl_2) 1795cm^{-1}. 2-Tetrahydrofuroyl chloride in ether (25ml) and dichloromethane (10ml) was added dropwise to an ice bath cooled solution of diazomethane (ca 80mmol) in ether (150ml). The reaction mixture was stirred 0.25h then a stream of hydrogen chloride gas passed into the solution for ca 2 minutes then stirred a further 0.25h, washed with saturated brine, dried, concentrated and flash chromatographed on silica gel eluting with 5,7.5 and 10% ethyl acetate in hexane to provide the title compound (2.46g, 41%); (Found: M^+, 148.0279. $C_6H\ ClO_2$ requires M, 148.0291); ν_{max}(CH_2Cl_2) 1739, 1395, 1071 and 936cm^{-1}; δ_H($CDCl_3$, 250MHz) 1.8–2.4 (4H, m) and 3.9–4.6 (5H, m).

(b) (3R,4R)-3-Phenoxyacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-one

(RS)-2-Chloroacetyltetrahydrofuran (2.46g, 16.5mmol), (3R,4R)-4-mercapto-3-phenoxyacetamidoazetidin-2-one (4.157g, 16.5mmol) and potassium carbonate (2.227g 16.5mmol) in DMF (10ml) were stirred for 2h, diluted with ethyl acetate, washed twice with water and with brine, dried concentrated and flash chromatographed eluting with 40,30, 20,10 and 0% hexane in ethyl acetate to give the title compound as a foam (3.547g, 59%); ν_{max}(CH_2Cl_2) 3405, 1785, 1693, 1520, 1496 and 1240cm^{-1}; δ($CDCl_3$, 250MHz) 1.9–2.3 (4H, m), 3.42 and 3.62, 3.46 and 3.56 (together 2H, 2 ABq, J15.8Hz, 15.4Hz), 3.85–4.0 (2H, m), 4.4–4.5 (1H, m), 4.58 (2H, s), 5.01, 5.04 (together 1H, 2d, J4.7Hz), 5.59 (1H, dd, J 8.8, 4.5Hz) 6.62, 6.68 (together 1H, 2s), 6.9–7.4 (5H, m) and 7.45, 7.47 (together 1H, 2d, J8.8Hz). [Mass spectrum: M^+(364)].

(c) t-Butyl (RS)-2-Hydroxy-2-[(3R,4R)-3-phenoxyacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate

0.5M t-Butyl glyoxylate in 1,2-dichloroethane (20ml) and triethylamine (140$1\mu l$, 1mmol) were added to (3R,4R)-phenoxyacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-one (3.547g, 9.7mmol) in 1,2-dichloroethane (10ml). The mixture was stirred 1h, concentrated in vacuo and flash chromatographed eluting with 50,60,70% ethyl acetate in hexane (3.663g, 76%); ν_{max}(CH_2Cl_2) 3471, 3407, 1782, 1736, 1692, 1521, 1290, 1154 and 1083cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.53 (9H, s), 1.85–2.25 (4H, m), 3.4–3.7 (2H, m), 3.8–4.0 (2H, m), 4.3–4.45 (1H, m), 4.57 (2H, s), 5.07, 5.09, 5.16, 5.18 (together 1H, 4d, J4.8Hz), 5.25–5.45 (1H, m), 5.48, 5.58 (together 1H, 2dd, J4.8, 8.8Hz), 6.9–7.4 (5H, m) and 7.41, 7.56 (together 1H, 2d, J8.7Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+(495)].

(d) t-Butyl 2-[(3R,4R)-3-Phenoxyacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio] azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

Thionyl chloride (0.81ml, 11.1mmol) in THF (5ml) was added dropwise to the hydroxy compound (3.663g, 7.4mmol) and 2,6-lutidine (1.29ml, 11.1mmol) in THF (15ml) at −20° C. The mixture was stirred 0.5h, filtered and the filtrate evaporated in vacuo, toluene added and re-evaporated to give t-butyl(RS)-2-chloro-2-[(3R,4R)-3-phenoxyacetamido-4-[(RS) -tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate as a foam (4.222g).

To the crude chloro compound in dioxan (10ml) was added tri-n-butylphosphine (4.06ml, 16.3mmol), the solution stirred 0.75h, [Bdiluted with ethyl acetate, washed with dilute sodium hydrogen carbonate solution, water and brine, dried concentrated and flash chromatographed on silica gel eluting with 30,40,50,60,70,80% ethyl acetate in hexane to give the title compound as a foam (3.827g, 76%); ν_{max} (CH_2Cl_2) 3417, 1764, 1731, 1690, 1628, 1523, 1171 and 1082cm^{-1} [Mass spectrum: +ve ion (thioglycerol) MH^+ (679)].

(e) t-Butyl (6R,7R)-7-Phenoxyacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The phosphorane (3.827g) and benzoic acid (20mg) in toluene (75ml) were purged with argon then heated under argon in an oil bath at 130° C. for 6h. The solution was left to cool and flash chromatographed on silica gel eluting with 30% ethyl acetate in hexane to give the title compound as a foam (2.267g, 87%); ν_{max}(CH_2Cl_2) 3406, 1785, 1697, 1519, 1155 and 1054cm^{-1}; δ_H($CDCl_3$, 250MHz) 1.53, 1.54

(together 9H, 2s), 1.5–2.5 (4H, m), 3.29 and 3.61, 3.39 and 3.56 (together 2H, 2ABq, J18.6, 18.0Hz), 3.8–4.0 (2H, m), 4.57 (2H, s), 4.9–5.0, 5.05–5.2 (together 1H, 2m), 5.01, 5.02 (together 1H, 2d, J4.8Hz), 5.84, 5.91 (together 1H, 2dd, J4.8, 9.4Hz) and 6.9–7.4 (6H, m). [Mass spectrum +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(483)].

(f) t-Butyl (6R,7R)-7-Amino-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

Phosphorus pentachloride (1.538g, 7.5mmol) in dichloromethane (39ml) was added to t-butyl(6R,7R)-7-phenoxyacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (2.267g, 4.9mmol) and N-methylmorpholine (1.1ml, 10mmol) in dichloromethane (20ml) at −25° C. The reaction was stirred at −10±5° C. for 0.75h then methanol (10ml) added all at once, stirred 0.75h then water (20ml) added and stirred vigorously for 1h. The dichloromethane was evaporated in vacuo, the aqueous residue washed with ether then adjusted to pH7 with ammonium hydroxide in the presence of ethyl acetate. The mixture was extracted twice with ethyl acetate, the extracts dried, concentrated and flash chromatographed on silica gel eluting with 30,40,50% ethyl acetate in hexane to give the more mobile (S)-diastereoisomer of the title compound (0.431g, 27%); (Found: M^+, 326.1299. $C_{15}H_{22}N_2O_4S$ requires M, 326.1300); $\nu_{max}(CH_2Cl_2)$ 1777, 1716, 1158 and 1052cm^{-1}; $\delta_H(CDCl_3$, 250MHz) 1.52 (9H,s), 1.55–1.8 (1H, m), 1.85–2.05 (4H, m), 2.3–2.45 (1H, m), 3.30 and 3.59 (2H, ABq, J18.4Hz), 3.8–4.0(2H,m), 4.75 (1H, d, J5.0Hz) and 4.9–5.0 (2H, m). Further elution with 60% ethyl acetate in hexane gave the more polar (R)-diastereoisomer (0.533g, 33%); (Found: M^+326.1299. $C_{15}H_{22}N_2O_4S$ requires M, 326.1300) ν_{max} (CH_2Cl_2) 1776, 1721, 1158 and 1052cm^{-1}; $\delta_H(CDCl_3$, 250MHz) 1.41 (2H, bs), 1.54 (9H, s), 1.6–1.85 (1H, m), 1.9–2.05 (2H, m), 2.05–2.2 (1H, m), 3.40 and 3.55 (2H, ABq, J17.8Hz) 3.8–4.0 (2H, m), 4.67 (1H, d, J5.0Hz), 4.93 (1H, d, J5.0Hz), 5.0–5.15 (1H, m).

(g) t-Butyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-(tetrahydrofuran-2-yl)-ceph-3-em-4-carboxylate

Mesyl chloride (141μl, 1.8mmol) was added to 2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid hydrochloride (0.744g, 1.65mmol) and N,N-diisopropylethylamine (576μl, 3.3mmol) in DMF (5ml) at −40° C. The reaction mixture was stirred 0.5h at −30±10° C. then t-butyl (6R,7R)-7-amino-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate, more mobile diastereoisomer (0.431g, 1.3mmol) in DMF (5ml) followed by pyridine (1471μl, 1.8mmol) were added. Stirred 1h without further cooling then diluted with ethyl acetate, washed twice with water and with brine, dried, concentrated and flash chromatographed on silica gel eluting with 30,35 and 40% ethyl acetate in hexane to give the title compound as a foam (0.83g, 84%); $\nu_{max}(CH_2Cl_2)$ 3396, 3277, 1782, 1732, 1683, 1526, 1248, 1156 and 1051cm^{-1}; $\delta_H[(CD_3)_2SO$, 250MHz]1.47 (9H, s), 1.55–1.75 (1H, m), 1.8–2.0 (2H, m), 2.05–2.2 (1H, m), 3.44 and 3.50 (2H, ABq, J18.3Hz), 3.65–3.95 (2H, m), 3.81 (3H, s), 4.6–4.7 (1H, m), 5.14 (1H, d, J4.8Hz), 5.66 (1H, dd, J4.8, 7.9Hz), 6.70 (1H, s), 7.2–7.4 (15H, m), 8.88 (1H, s) and 9.54 (1H, d, J7.9Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(774)].

(h) Sodium (6R-7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

t-Butyl (6R,7R)-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-(tetrahydrofuran-2-yl) ceph-3-em-4-carboxylate, single diastereoisomer (0.832g, 1.1mol) in 0.1M hydrochloric acid in 90% formic acid (11ml) was stood for 1h, concentrated hydrochloric acid (200μl) added and left for a further 1.5h then evaporated to dryness in vacuo. The residue in water (ca 5ml) was adjusted to pH6.5 with 1M sodium hydroxide solution and chromatographed on HP20SS eluting with 0,1,2 and 3% THF in water. Fractions containing the product, h.p.l.c. analysis, were combined, concentrated and freeze dried to give the title compound as a mixture of diastereoisomers (271mg, 52%); ν_{max}(KBr) 1762, 1669, 1603, 1530, 1388 and 1039cm^{-1}; $\delta_H[(CD_3)_2SO$, 250MHz] 1.4–2.05 (4H, m), 3.19 and 3.36, 3.26 and 3.83 (together 2H, 2ABq, J17.5, 16.8Hz), 3.55–3.85 (1H, m), 3.83 (3H, s), 4.85–4.95, 5.15–5.25 (together 2H, 2m), 4.96, 4.97 (together 1H, 2d, J4.7Hz), 5.49, 5.53 (together 1H, 2dd, J4.7, 7.9Hz), 6.74, 6.75 (together 1H, 2s), 7.24 (2H, s), 9.49, 9.52 (together 1H, 2d, J7.9Hz) [Mass spectrum +ve ion (thioglycerol) MH^+(476), MNa^+(498)].

The same mixture of diastereoisomers was obtained by progressing the other diastereoisomer isolated in stage (f).

EXAMPLE 2

Pivaloyloxymethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Pivaloyloxymethyl bromide (0.15g) and sodium iodide (0.15g) in acetone (1ml) were stirred 0.5h, filtered and the filtrate evaporated to give the iodide. This in toluene (0.5ml) was added to sodium (6R,7R)-7-[2-(-2-aminothiazol-4- yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (0.191g) in N-methylpyrrolidone (1ml) and stirred 0.5h. The reaction mixture was diluted with ethyl acetate, washed twice with water and with brine, dried, concentrated and flash chromatographed on silica gel eluting with 80% ethyl acetate in hexane to give the title compound (130mg, 57%); ν_{max} (CH_2Cl_2) 3478, 3391, 1787, 1752, 1685, 1125, 1098 and 1052cm−1; $\delta_H(CDCl_3$, 250MHz), 1.23 (9H,s), 1.6–2.5 (4H, m), 3.37 and 3.66, 3.43 and 3.62 (together 2H, 2ABq, J18.8, 17.8Hz), 3.8–4.05 (2H, m), 4.10 (3H, s), 4.85–5.0, 5.15–5.25 (together 1H, 2m), 5.07, 5.08 (together 1H, 2d, J4.8, 4.7Hz), 5.8–6.05 (3H, m), 6.95, 6.96 (together 1H, 2s) and 7.54, 7.65 (together 1H, 2d, J8.8, 8.5Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+568)].

EXAMPLE 3

Sodium (6R.7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]-ceph-3-em-4-carboxylate

(a) Tetrahydropyran-2-carboxylic acid

3,4-Dihydro-2H-pyran-2-carboxylic acid, sodium salt (5.0g) in water (30ml) was treated with 10% Palladium on carbon catalyst (0.2g) and the mixture hydrogenated until there was no further uptake of hydrogen. The mixture was filtered through Kieselguhr, the filtrate passed through a column of 'Amberlite IR120(H^+), evaporated in vacuo and the residue dissolved in dichloromethane, dried and evaporated to give the title compound as colourless oil. (3.3g, 76%); (Found: M^+, 130.0631. $C_6H_{10}O_3$ requires M, 130.0630; $\nu_{max}(CH_2Cl)$ 3500-2750 (v.br), 1772, 1725cm^{-1}; $\delta_H(CDCl_3)$, 1.5–1.7 (4H, m), 1.8–2.1 (2H, m), 3.50–3.59 (1H, m), 3.99–4.14 (2H, m) and 7.28 (1H, br.s).

(b) 2-(2-Chloroacetyl)tetrahydropyran

Tetrahydropyran-2-carboxylic acid (3.3g) in dry dichloromethane (60ml) was treated with oxalyl chloride (4.8g,

3.3ml) and DMF (2–3 drops). After the initial effervescence had ceased the mixture was left for a further 1h at ambient temperature. The solvent and excess oxalyl chloride were removed in vacuo and the resultant oil [$\nu_{max}(CH_2Cl_2)$ 1830cm^{-1}] was dissolved in dichloromethane (20ml). This acid chloride solution was then added dropwise to a freshly prepared ethereal solution of diazomethane (ca 2 fold excess) cooled to 0–5° C., t.l.c. analysis (60% ethyl acetate in hexane) showed a single mobile spot, i.r. spectrum of a sample showed clean conversion to the diazoketone [ν_{max} (CH_2Cl_2) 2100cm^{-1}]. Hydrogen chloride gas was bubbled through the solution until no further starting material was observed by t.l.c. The mixture was washed with brine, dried and the solvent removed in vacuo and the residue purified by flash chromatography on silica gel. The title compound was obtained as a pale yellow oil, (2.8g, 68%); $\nu_{max}(CH_2Cl_2)$ 1740cm^{-1}; $\delta_H(CDCl_3)$ 1.4–1.7 (4H, m), 1.91–1.98 (2H, m), 3.42–3.53 (1H, m), 3.95–4.07 (2H, m) and 4.48 (2H, s) [Mass spectrum: +ve ion (NH_3), MH^+(163), MNH_4^+(180)].

(c) (3R,4R)-3-Phenylacetamido-4-[(RS)-tetrahydropyran-2-ylcarbonylmethylthio]azetidin-2-one

3R,4R-Mercapto-3-phenylacetamidoazetidin-2-one (2.6g) and 2-(2-chloroacetyl)tetrahydropyran (1.6g) in DMF (20ml) were treated with potassium carbonate (1.6g) at ambient temperature for ca 2h until t.l.c. (80% ethylacetate in hexane) showed loss of starting material. The reaction mixture was diluted with ethyl acetate,washed with water (x3), brine, dried and concentrated. The title compound was obtained by flash chromatography (60%,70% ethyl acetate in hexane, ethyl acetate) as a mixture of diastereoisomers as a colourless foam (1.7g, 70%); $\nu_{max}(CH_2Cl_2)$, 3380(w), 1783, 1726, 1684cm^{-1}; $\delta_H(CDCl_3)$ 1.3–1.7 (4H, m), 1.8–2.0 (2H, m), 3.3–3.6 (3H, m), 3.66 (2H, s), 3.86–3.90 (1H, m), 4.03–4.07 (1H, m), 4.92 (1H, d, J4.6Hz), 5.51 (1H, dd, J4.4, 8.6Hz) 6.42 (d, J8.7Hz), 6.48, 6.51 (together 1H, 2s) and 7.27–7.36 (5H, m). [Mass spectrum: M^+(362)].

(d) t-Butyl (RS)-2-Hydroxy-2-[(3R,4R)-3-phenylacetamido-4-[(RS)-tetrahydropyran-2-ylcarbonylmethylthio]-azetidin-2-on-1-yl]acetate

(3R,4R)-3-Phenylacetamido-4-[(RS)-tetrahydropyran-2-yl-carbonylmethylthio]azetidin-2-one (1.7g) in 1,2-dichloroethane (20ml) was successively treated with 0.5M t-butyl glyoxylate in 1,2-dichlorethane (10ml) and triethylamine (50mg, 70μl) and monitored by t.l.c. (ethyl acetate) until no starting material remained. The reaction mixture was concentrated and flash chromatography (70% ethyl acetate in hexane, ethyl acetate) to afford the title compound as a yellow foam (1.9g, 82%); ν_{max} (CH_2Cl_3) 3400 (w), 1780, 1736, 1687cm^{-1}; $\delta_H(CDCl_3)$ 1.49 (9H, s) overlapping 1.44–1.61 (4H, m), 1.8–2.0 (2H, m), 3.35–3.58 (3H, m), 3.65 (2H, s), 3.81–3.92 (1H, m), 4.01–4.06 (1H, m), 4.28–4.43 (1H, m), 4.99, 5.00, 5.07 (together 1H, 3d, J4.7Hz), 5.21, 5.32, 5.33 (together 1H, 3d, J6.8, 7.7, 7.6Hz), 5.42, 5.50 (together 1H, 2dd, J4.8, 8.7Hz, 6.35, 6.36, 6.61 (together 1H, 3d, J8.7Hz) and 7.27–7.38 (5H, m).

(e) t-Butyl 2-[(3R,4R)-3-Phenylacetamido-4-[(RS)-tetrahydropyran-2-ylcarbonylmethylthiolazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

t-Butyl 2-hydroxy-2-[(3R,4R)-3-phenylacetamido-4-[(RS)-tetrahydropyran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate (1.9g) in dry THF (10ml) was treated with 2,6-lutidine (0.62g, 0.67ml) followed by thionyl chloride (0.69g, 0.42ml) in THF (5ml) dropwise at <–20° C. under argon. The reaction mixture was allowed to warm slowly to ca 0° C. at which point no starting material was observed by t.l.c. (ethyl acetate). The reaction mixture was filtered and solvent removed in vacuo, the residue dissolved in toluene and evaporated to afford crude t-butyl (RS)-2-chloro-2-((3R, 4R)-3-phenylacetamido-4-[(RS)-tetrahydropyran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate as a brown gum. This was dissolved in dry dioxan (10ml) and treated with tri-n-butylphosphine (1.79g, 2.2ml). The reaction mixture was stirred until loss of starting material was observed by t.l.c. (ethyl acetate) ca 0.5h. After removal of solvent in vacuo the title compound was obtained by flash chromatography (eluting with 50,60,80% ethyl acetate in hexane, ethyl acetate) as a pale brown foam (1.95g, 75%); $\nu_{max}(CH_2Cl_2)$ 3417(w), 1762, 1681, 1625cm^{-1}. [Mass spectrum +ve ion (thioglycerol) MH^+(677)].

(f) t-Butyl (6R,7R)-7-Phenylacetamido-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate

t-Butyl 2-[(3R, 4R)-3-phenylacetamido-4-[(RS)-tetrahydropyran-2-yl]carbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate (1.95g) in dry toluene (50ml) was refluxed for 8h under argon. The solvent was removed in vacuo and the title compound obtained by flash chromatography (30% ethyl acetate in dichloromethane) as a yellow foam (l.15g, 87%); ν_{max} (CH_2Cl_2), 3415(w), 1783, 1721, 1687cm^{-1}; $\delta_H(CDCl_3)$, 1.54, 1.56 (together 9H, 2s) overlapping 1.46–1.68 (4H, m), 1.76–1.94 (2H, m), 3.42–3.68 (5H, m), 3.97–4.06 (2H, m), 4.52–4.65 (1H, m), 4.98 (1H, d, J4.8Hz), 5.68, 5.71 (together 1H, dd, J4.7) and 7.23–7.36 (5H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa+(481)].

(g) t-Butyl (6R,7R)-7-Amino-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate

t-Butyl (6R, 7R) -7-phenylacetamido-3- [(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate (1.1g) in dry dichloromethane (50ml) at –20° C. under argon was successively treated with N-methylmorpholine (0.55g, 0.6ml) and phosphorus pentachloride (0.65g as 16.25ml of a 40mg/ml solution in dry dichloromethane) and stirred at –20° C. for 0.75h. Methanol (50ml) was added and reaction mixture allowed to warm to ambient temperature over a period of ca 0.5h. Water (50ml) was added and reaction mixture stirred vigourously for a further 0.5h. The dichloromethane was removed in vacuo, ethyl acetate added and aqueous layer adjusted to pH8 with 0.880 ammonia and reextracted with ethyl acetate. The organic extracts were washed with water, brine, dried, concentrated and flash chromatographed on silica gel (eluting with 70,80% ethyl acetate in hexane, ethyl acetate) . The first isomer to be eluted (isomer A) was obtained as a white foam (300mg, 37%); ν_{max} (CH_2Cl_2) 1776, 1717cm^{-1}; $\delta_H(CDCl_3)$, 1.53 (9H, s) overlapping 1.4–1.7 (4H, m), 1.73–1.97 (2H, m), 3.35–3.55 (1H, m) overlapping 3.49 and 3.55 (2H, ABq, J18.4Hz), 3.96–4.00 (1H, m), 4.51–4.55 (1H, m), 4.72 (1H, d, J5.0Hz) and 4.93 (1H, d, J5.0Hz). [Mass spectrum: M^+(340)]. The second isomer to be eluted (isomer B) was obtained as a white foam (400mg, 49%); ν_{max} (CH_2Cl_2), 1715, 1721cm^{-1}; $\delta_H(CDCl_3)$ 1.56 (9H, s) overlapping 1.49–1.66 (4H, m), 1.84–2.05 (2H, m), 3.44 and 3.62 (2H, ABq, J 17.8) overlapping 3.45–3.54 (1H, m), 4.01–4.11 (1H, m), 4.56–4.61 (1H, m), 4.69 (1H, d, J5.0Hz) and 4.93 (1H, d, J5.0Hz). [Mass spectrum: M^+(340)].

(h) t-Butyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[tetrahydropyran-2-yl]ceph-3-em-4-carboxylate

Mesyl chloride (121mg, 82μl) was added to 2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid hydrochloride (466mg) and N,N-diisopropylethylamine (252mg, 340μl) in dry DMF (10ml) at −50° C. under argon and stirred at −50° C. for 1h. Then t-butyl (6R,7R)-7amino-3-(tetrahydropyran-2-yl)-ceph-3-em-4-carboxylate (Isomer A, 300mg) in dry DMF (5ml) followed by pyridine (70mg, 72μl) were added and reaction mixture left for a further 1h whilst warming to ambient temperature. The reaction mixture was partitioned between ethyl acetate and water, reextracted with ethyl acetate, organic extracts washed with water (x3) and brine, dried, concentrated and flash chromatography (eluting with 30,40,50,60% ethyl acetate in hexane) to afford the title compound as a pale yellow foam (420mg, 62%); $\nu_{max}(CH_2Cl_2)$ 3420, 1784, 1732 (shoulder), 1717, 1685cm^{-1}; $\delta_H(CDCl_3)$, 1.53 (9H, s) overlapping 1.4–1.7 (4H, m), 1.73–1.94 (2H, m), 3.38–3.58 (3H, m), 3.95–4.00 (1H, m), 4.07 (3H, s), 4.54–4.59 (1H, m), 5.02 (1H, d, J4.8Hz), 5.90 (1H, dd, J4.5, 9.1Hz) 6.74 (1H, s), 6.86 (1H, d, J8.8Hz), 7.04 (1H, s) and 7.30 (15H, s). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(788)].

(i) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate

t-Butyl (6R,7R)-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-(tetrahydropyran-2-yl)ceph-3-em-4-carboxylate (400mg) was dissolved in 0.1M hydrochloric acid in 90% formic acid (5.22ml) and set aside for 0.5h, concentrated hydrochloric acid (50μl) added and left for a further 1.5h. The mixture was evaporated in vacuo, diluted with water, adjusted to pH6.7 with sodium bicarbonate, then chromatographed on HP20SS eluting with water then 1,2,4,6,8% THF in water. Fractions containing a diastereoisomeric mixture of the title compound (h.p.l.c.) were concentrated in vacuo and freeze dried (170mg, 66%); ν_{max} (KBr) 1770, 1670, 1600, 1535cm^{-1}; $\delta_H[(CD_3)_2SO]$, 1.3–1.5 (4H, m), 1.6–1.85 (2H, m), 3.24–3.44 (m, masked by HOD peak), 3.83 (3H, s) overlapping 3.76–3.95 (1H, m), 4.46–4.50, 4.82–4.86 (together 1H, 2m), 4.94 (1H, d, J4.7Hz), 5.46–5.53 (1H, m), 6.74, 6.75 (together 1H, 2s), 7.23 (2H, s) and 9.48, 9.51 (together 1H, 2d, J5.6, 5.5Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+(490), MNa^+ (512)].

The second isomer eluted (isomer B) in step (g) (400mg) was progressed through step (h) as before yielding a pale yellow foam (550mg, 61%); $\nu_{max}(CH_2Cl_2)$, 3420, 1783, 1729, 1687cm^{-1}; $\delta_H(CDCl_3)$ 1.55 (9H, s) overlapping 1.44–1.68 (4H, m), 1.82–1.96 (2H, m), 3.44 and 3.65 (2H, ABq, J18.0) overlapping 3.42–3.58 (1H, m), 4.07 (3H, s) overlapping 3.96–4.10 (1H, m), 4.66–4.69 (1H, m), 4.66–4.69 (1H, m), 5.01 (1H, d, J4.7Hz), 5.86 (1H, dd, J4.8, 8.9Hz), 6.75 (1H, s) overlapping 6.75–6.78 (1H, m), 7.01 (1H, s) and 7.30 (15H, s). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(788)]. This was then progressed through step (i) to afford the same mixture of diastereoisomers.

EXAMPLE 4

Pivaloyloxymethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate

The title compound was prepared from the compound of Example 3 as described in Example 2 and obtained as a pale yellow foam (59%); $\nu_{max}(CH_2Cl_2)$; 3388, 1787, 1752, 1688cm^{-1}; $\delta_H(CDCl_3)$ 1.24 (9H, s), 1.42–1.64 (4H, m), 1.74–1.90 (2H, m), 3.40–3.75 (3H, m), 4.07, 4.08 (together 3H, 2s) overlapping 3.96–4.10 (1H, m), 4.56–4.59, 4.80–4.83 (together 1H, 2m), 5.07, 5.08 (together 1H, 2d, J4.8, 4.7Hz), 5.66 (2H, br.s), 5.85–6.03 (3H, m), 6.86, 6.89 (together 1H, 2s) and 7.59 (1H, d, J8.9Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+(582); MNa^+(604)].

EXAMPLE 5

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylic acid

(a) t-Butyl (6R,7R)-3-[(R)-tetrahydrofuran-2-yl]-7-[2-(2-tritylaminothiazol-4-yl)-2-(Z)-trityloxyiminoacetamido]-ceph-3-em-4-carboxylate

Methanesulphonyl chloride (96μl, 1.25mmol) was added to sodium 2-(2-tritylaminothiazol-4-yl)-2-(Z)-trityloxyiminoacetate (852mg, 1.2mmol) in DMF (2ml) at <−40° C. The mixture was stirred 0.5h at −30±10° C. then t-butyl (6R,7R)-7-amino-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (326mg, 1mmol) in DMF (2ml), followed by pyridine (101μl, 1.25mmol), were added. The reaction was stirred for 1h without further cooling then diluted with ethyl acetate, washed twice with water and with brine, dried, concentrated in vacuo and flash chromatographed eluting with 25, 30% ethyl acetate in hexane to give the title compound as a colourless foam (665mg, 68%); ν_{max} (CH_2Cl_2) 3395, 1787, 1722, 1687, 1527, 1449, 1156 and 1051cm^{-1}; δ_H ($CDCl_3/CD_3OH$) 1.55 (9H, s), 1.65–2.25 (4H, m), 3.32 and 3.40 (2H, ABq, J 17.6Hz), 3.8–4.0 (2H, m), 5.08 (1H, d, J 4.8Hz), 5.13 (1H, dd, J 7.0, 8.1Hz), 5.91 (1H, d, J 4.5Hz), 6.56 (1H, s), 7.2–7.5 (30H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+ (1002)].

(b) (6R)7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylic acid

t-Butyl (6R,7R)-3-[(R)-tetrahydrofuran-2-yl]-7-[2-(2-tritylaminothiazol-4-yl)-2-(Z)-trityloxyiminoacetamido] ceph-3-em-4-carboxylate (660mg) was dissolved in 0.1M hydrochloric acid in 90% formic acid (7ml) and left for 1h then concentrated hydrochloric acid (250μl) was added and left a further 0.75h. The mixture was evaporated to dryness in vacuo, the residue diluted with water, adjusted to pH3.2 with 0.25M sodium hydroxide then chromatographed on HP20SS eluting with 0 to 15% THF in water. The fractions containing the title compound (h.p.l.c. analysis) were combined, concentrated and freeze-dried to give a colourless solid (102mg, 35%); ν_{max} (CH_2Cl_2) 3315, 1763, 1663, 1626, 1178 and 1045cm^{-1}; $\delta_H[(CD_3)_2SO]$1.6–2.2 (4H, m), 3.35–3.9 (4H, m), 4.73, 4.95 (together 1H, 2dd, J 8.3, 9.1Hz), 5.13, 5.15 (together 1H, 2d, J 4.6Hz), 5.7–5.8 (1H, m), 6.66, 6.68 (together 1H, 2s), 7.14 (2H, s), 9.44, 9.48 (1H, 2d, J 7.9Hz), 11.30, 11.31 (together 1H, 2s). [Mass spectrum: +ve ion (thioglycerol) MH^+(440)].

EXAMPLE 6

Diastereoisomers of (6R, 7R) -7-Amino-3-(tetrahydrofuran-2-yl) ceph-3-em-4-carboxylate

(a) (RS) -2-Bromoacetyltetrahydrofuran

A stream of diazomethane (from N-methyl-N-nitrosotoluene-4-sulphonamide, 18.0g) in argon (P.

Lombardi, *Chem. and Ind.*, 1990, (21), 708) was passed into a solution of (RS)-tetrahydrofuroyl chloride [prepared from (RS)-tetrahydrofuroic acid (3.48g, 30mmol) as described in Example 2(*a*)] in dichloromethane (60ml) cooled in an ice bath. When the diazomethane addition was complete, 48% aqueous hydrogen bromide (5.6ml, 33.2mmol) was added. The mixture was stirred 0.25h then washed twice with water, dried, concentrated and flash chromaographed on silica gel eluting with 10% ethyl acetate in hexane to give the title compound as a pale yellow oil (4.44g, 77%); ν_{max} (CH_2Cl_2) 1733, 1245, 1073 and 938cm^{-1}; δ_H ($CDCl_3$) 1.85–2.35 (4H, m), 3.85–4.05 (2H, m), 4.20 (2H, s), 4.54 (1H, dd, J 6.1, 8.2Hz). [Mass spectrum: +ve ion (ammonia) MNH_4^+(210)].

(b) 4-Methoxybenzyl (2RS)-2-hydroxy-2-[(3R, 4R)-3-phenylacetamido-4- [(RS) -tetrahydrofuran-2-yl-carbonylmethylthio]-azetidin-2-on-1-yl]acetate

Toluene-4-sulphonic acid (6.0g, 31.5mmol) in water (15ml) was added to a solution of 4-methoxybenzyl (2RS)-2-hydroxy-2-[(1R,5R)-3-benzyl-4-thia-2,6-diazabicyclo [3.2.0]hept-2-en-7-on-6-yl]acetate (7.42g, 18.0mmol) prepared from Penicillin G as described for the benzhydryl ester derived from Penicillin V, S. Yamamoto, N. Haga, T. Aoki, S. Hayashi, H. Tanida, and W. Nagata, *Heterocycles*, 1977, 8, 283) in dichloromethane (30ml) and acetone (30ml) . After stirring for 2.5 h at room temperature, the reaction mixture was diluted with dichloromethane, washed with water (x2), dried and concentrated in vacuo to yield crude 4-methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-mercapto-3-phenylacetamidoazetidin-2-on-1-yl]acetate as a yellow foam.

The crude thiol was dissolved in acetone (35ml) and treated with a solution of (RS)-2-bromoacetyltetrahydrofuran (3.48g, 18.0mmol) in acetone (5ml). After 10 min, potassium carbonate (1.24g, 8.9mmol) was added, and the mixture stirred for a further 30 min. The reaction mixture was diluted with ethyl acetate, washed successively with water (x2) and brine, dried and concentrated. The residue was flash chromatographed on silica gel eluting with 50, 70 and 80% ethyl acetate in hexane yielding the title compound as a colourless foam (5.40g, 55%); ν_{max} (CH_2Cl_2) 3409, 1781, 1745, 1684, 1613, 1516cm^{-1}. [Mass spectrum +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(565)].

(c) 4-Methoxybenzyl 2-[(3R,4R)-3-phenylacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

A solution of thionyl chloride (1.36ml, 18.6mmol) in THF (10ml) was added dropwise to the hydroxy compound (6.72g, 12.4mmol) and 2,6-lutidine (2.16ml, 18.6mmol) in THF (30ml) at –20° C. After stirring for 1h, the reaction mixture was filtered through a pad of celite, and the filtrate evaporated in vacuo. Toluene was added and re-evaporated to yield 4-methoxybenzyl (RS)-2-chloro-2-[(3R,4R)-3-phenylacetamido-4-[(RS)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate as an oil.

The crude chloro compound was dissolved in dioxan (30ml) and treated with tri-n-butylphosphine (6.8ml, 27.3mmol). After stirring for 30 min. at room temperature, the reaction mixture was diluted with ethyl acetate and washed successively with dilute sodium hydrogen carbonate solution, water and brine. The organic solution was dried, concentrated and then flash chromatographed on silica gel eluting with 50, then 80% ethyl acetate in hexane to give the title compound as a foam (6.54g, 73%); ν_{max} (CH_2Cl_2) 3422, 1763, 1732, 1680, 1613, 1515, 1174cm^{-1}. [Mass spectrum +ve ion/thioglycerol) MH^+(727), MNa^+(749)].

(d) 4-Methoxybenzyl (6R,7R)-7-phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of the phosphorane (6.40g, 8.82mmol) and benzoic acid (20mg) in toluene (100ml) was heated in an oil bath at 130° C. for 10h under argon. The reaction mixture was cooled, concentrated and the residue purified by chromatography on silica gel eluting with 20, 30, 40, 50% ethyl acetate in hexane yielding the title compound as a yellow oil (3.50g, 78% yield); ν_{max} (CH_2Cl_2) 3411, 1783, 1723, 1688, 1515cm^{-1}; δ_H($CDCl_3$, 250MHz) 1.50–2.39 (together 4H, m), 3.27 and 3.60, 3.32 and 3.49 (together 2H, 2ABq, J 18.7, 17.9Hz), 3.58 and 3.70, 3.63 and 3.73 (together 2H, 2ABq, J 16.2, 16.1Hz), 3.80, 3.82 (together 3H, 2S), 3.84–3.97 (together 2H, 2m), 4.91, 5.18 (together 1H, 2m), 4.90, 4.94 (together 1H, 2d, J 4.7Hz), 5.17 (2H, s), 5.73, 5.82 (together 1H, 2dd, J 9.1, 4.7Hz), 5.98, 6.02 (together 1H, 2d, J 9.1Hz), 6.87 and 6.90 (together 2H, 2d, J 8.6Hz), 7.23–7.40 (7H, m). [Mass spectrum +ve ion (3-nitrobenzylalcohol, sodium acetate) MNa^+(531)].

(e) 4-Methoxybenzyl (6R,7R)-7-amino-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

Phosphorus pentachloride (2.15g, 10.32mmol) in dichloromethane (108ml) was added to 4-methoxybenzyl (6R,7R)-7-phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (3.40g, 6.69mmol) and N-methylmorpholine (1.50 ml, 13.64mmol) in dichloromethane (30ml) at –25° C. The reaction was stirred at –10±5° C. for 45min., then methanol (14ml) was added, and stirring continued for 45min. at room temperature. Water (27ml) was then added, and the mixture vigorously stirred for a further 1h. The dichloromethane was evaporated in vacuo, the aqueous residue washed with diethyl ether, then adjusted to pH7 with ammonium hydroxide in the presence of ethyl acetate. The mixture was extracted with ethyl acetate (x2), dried and concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with 50, 70, 80% ethyl acetate in hexane yielding 4-methoxybenzyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (980mg, 38%) as a yellow foam; ν_{max} (CH_2Cl_2) 1777, 1721, 1613, 1516, 1152cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.53–1.71 (1H, m), 1.84–2.02 (4H, m, 2 exch.), 2.25–2.40 (1H, m), 3.31 and 3.60 (2H, ABq, J 18.5Hz), 3.78–3.98 (2H, m), 3.81 (3H, s), 4.72 (1H, d, J 5.0Hz), 4.86–4.93 (2H, m), 5.19 (2H, s), 6.88 (2H, d, J 8.6Hz), 7.33 (2H, d, J 8.6Hz). [Mass spectrum: M^+(390)].

Further elution with ethyl acetate gave the more polar diastereoisomer, 4-methoxybenzyl (6R,7R)-7-amino-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (590mg, 23%). The product was recrystallised using ethyl acetate-hexane to yield an off-white solid m.p. 131–134° C. ν_{max} (CH_2Cl_2) 1775, 1726, 1613, 1516, 1156cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.58–1.70 (1H, m), 1.83–2.06 (4H, m, 2 exch.), 3.38 and 3.57 (2H, ABq, J 17.8HYz), 3.77–3.93 92H, m), 3.82 (3H, s), 4.68 (1H, d, J 4.9Hz), 4.92 (1H, d, J 4.9Hz), 5.07 (1H, m), 5.22 (2H, s), 6.90 (2H, d, J 8.6Hz), 7.35 (2H, d, J 8.6Hz). [Mass spectrum: M^+(390)].

EXAMPLE 7

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Methanesulphonyl chloride (203μl, 2.62mmol) was added to 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid

(528mg, 2.63mmol) and N,N-diisopropylethylamine (458μl, 2.63mmol) in DMF (8ml) at −30° C. After stirring at −30±10° C. for 30min., a solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (930mg, 2.38mmol) in DMF (5ml) was added, followed by pyridine (213 μl, 2.63mmol). The reaction mixture was transferred to an ice-bath and stirring continued for a further 1h. After dilution with ethyl acetate, the solution was washed successively with saturated sodium hydrogen carbonate solution, 5% aqueous citric acid, water (x2) and brine, dried and then concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with 50, 70 and 90% ethyl acetate in hexane to give the title compound as a yellow foam (1.138g, 83%); ν_{max} (CH_2Cl_2) 3389, 1783, 1732, 1682, 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.53–1.70 (1H, m), 1.88–2.01 (2H, m), 2.28–2.41 (1H, m), 3.33 and 3.62 (2H, ABq, J 18.7Hz), 3.79–3.98 (2H, m), 3.81 (3H, s), 4.08 (3H, s), 4.94 (1H, dd, J 9.0, 6.7Hz), 5.04 (1H, d, J 4.8Hz), 5.18 (2H, s), 5.88 (2H, br s, exch.), 5.98 (1H, dd, J 9.0, 4.8Hz), 6.90 (2H, d, J 8.6Hz), 6.94 (1H, s), 7.35 (2H, d, J 8.6Hz), 7.50 (1H, br. d, J 9.0Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(574)].

(b) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Aluminium chloride (162mg, 1.21mmol) was added to anisole (7ml) and dry dichloromethane (3.5ml) at −20° C. and stirred for 15 min. The temperature of the cooling bath was then lowered to −40° C. before addition of a solution of 4-methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (235mg, 0.41mmol) in dichloromethane (5ml). After 10 min., the solution was treated with trisodium citrate (0.5M, 12ml) and then vigorously stirred for 10 min at room temperature. The aqueous phase was separated, washed with dichloromethane (x2) and concentrated in vacuo. The residue was chromatographed on HP20SS eluting with water, then 1% THF in water. Fractions containing the product, (h.p.l.c. analysis), were combined and freeze-dried to give the title compound (126mg, 65%); ν_{max} (KBr) 3401, 1761, 1669, 1603, 1533, 1040cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1.43–1.59 (1H, m), 1.71–1.88 (2H, m), 2.0–2.12 (1H, m), 3.18 and 3.37 (2H, ABq, J 17.4Hz), 3.58 (1H, m), 3.78 (1H, m) , 3.81 (3H, s), 4.87 (1H, dd, J 8.7, 6.7Hz), 4.97 (1H, d, J 4.7Hz), 5.50 (1H, dd, J 8.1, 4.7Hz), 6.74 (1H, s), 7.21 (2H, br. s, exch.), 9.48 (1H, d, J 8.1Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$ (476), MNa$^+$(498)].

EXAMPLE 8

Pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamidol-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Pivaloyloxymethyl bromide (440mg, 2.26mmol) and sodium iodide (440mg, 2.93mmol) in acetone (3ml) were stirred for 30 min., filtered, and the filtrate concentrated in vacuo. The resulting iodide in toluene (2ml) was added to a solution of sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (560mg, 1.18mmol) in N-methylpyrrolidinone (5ml). After stirring for 45 min. at room temperature, the reaction mixture was diluted with ethyl acetate, washed successively with water (x3) and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel eluting with 80% ethyl acetate in hexane to give the title compound as a yellow foam (486mg, 73%); ν_{max} (CH_2Cl_2) 3390, 1776, 1749, 1681, 1532cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.22 (9H, s), 1.65 (1H, m), 1.99 (2H, m), 2.41 (1H, m), 3.37 and 3.68 (2H, ABq, J 18.8Hz), 3.80–4.01 (2H, m), 4.13 (3H, s), 4.92 (1H, dd, J 8.9, 6.8Hz), 5.08 (1H, d, J 4.8Hz), 5.85 and 5.92 (2H, ABq, J 5.6Hz), 5.98 (1H, dd, J 8.4, 4.8Hz), 6.07 (2H, br s, exch.), 7.03 (1H, s), 7.40 (1H, br. d, exch. J 8.4Hz). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(568)].

EXAMPLE 9

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl (6 R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylate

Methanesulphonyl chloride (198μl, 2.56mmol) was added to 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (515mg, 2.56mmol) and N,N-diisopropylethylamine (447μl, 2.57mmol) in DMF (8ml) at −30° C. After stirring at −30±10° C. for 30 min., a solution of 4-methoxybenzyl (6R, 7R) -7-amino-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (915mg, 2.35mmol) in DMF (5ml) was added, followed by pyridine (207μl, 2.56mmol). The reaction mixture was transferred to an ice-bath and stirring continued for a further 1.5h. After dilution with ethyl acetate, the solution was washed successively with saturated sodium hydrogen carbonate solution, 5% aqueous citric acid, water (x2) and brine, dried and then concentrated in vacuo. The residue was triturated several times with diethyl ether to yield the title compound as an off-white solid (1.06g, 79%); ν_{max} (CH_2Cl_2) 3390, 1783, 1730, 1687, 1606, 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.55–1.70 (1H, m), 1.86–1.98 (2H, m), 2.0–2.14 (1H, m), 3.40 and 3.59 (2H, ABq, J 17.8Hz), 3.78–3.93 (2H, m), 3.91 (3H, s), 4.12 (3H, s), 5.04 (1H, d, J 4.7Hz), 5.15 (1H, dd, J 7.7, 7.7Hz), 5.21 (2H, s), 5.87 (1H, dd, J 8.7, 4.7Hz), 6.55 (2H, br. s, exch.), 6.90 (2H, d, J 8.6Hz), 7.05 (1H, s), 7.36 (2H, d, J 8.6Hz), 7.65 (1H, br. d, J 8.7Hz). [Mass spectrum: +ve ion (thioglycerol) MH$^+$ (574)].

(b) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Aluminium chloride (740mg, 5.55mmol) was added to anisole (32ml) and dry dichloromethane (15ml) at −20° C. and stirred for 15 min. The temperature of the cooling bath was then lowered to −40° C. before addition of a solution of 4-methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (1.06 g, 1.85 mmol) in dichloromethane (10ml). After 10 min., the solution was treated with trisodium citrate (0.5M, 54ml) and then vigorously stirred for 10 min. at room temperature. The aqueous phase was separated, washed with dichloromethane (x2) and concentrated in vacuo. The residue was chromatographed on HP20SS eluting with water, then 1% THF in water. Fractions containing the product, (h.p.l.c. analysis), were combined and freeze-dried to give the title compound (560mg, 64%); ν_{max} (KBr) 3399, 1762, 1669, 1603, 1529, 1038cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1.50–1.91 (4H, m), 3.25 and 3.38 (2H, ABq, J 16.8Hz), 3.60–3.82 (2H, m), 3.84 (3H, s), 4.96 (1H, d, J 4.7Hz), 5.20 (1H, dd, J 8.6, 6.0Hz), 5.48 (1H, dd,

J 8.1, 4.7Hz), 6.76 (1H, s), 7.23 (2H, br. s, exch.), 9.50 (1H, d, J 8.1Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH^+(476), MNa^+(498)].

EXAMPLE 10

Pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Pivaloyloxymethyl bromide (247mg, 1.27mmol) and sodium iodide (247mg, 1.65mmol) in acetone (5ml) were stirred for 30 min., filtered, and the filtrate concentrated in vacuo. The resulting iodide in toluene (3ml) was added to a solution of sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (320mg, 0.67mmol) in N-methylpyrrolidinone (5ml). After stirring for 30 min. at room temperature, the reaction mixture was diluted with ethyl acetate, washed successively with water (x3) and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel eluting with 80% ethyl acetate in hexane to give the title compound as a yellow foam (297mg, 78%); ν_{max} (CH_2Cl_2) 3387, 1786, 1752, 1735, 1686, 1605cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.22 (9H, s), 1.69 (1H, m), 1.98 (2H, m), 2.18 (1H, m), 3.43 and 3.62 (2H, ABq, J 18.0Hz), 3.80–3.96 (2H, m), 4.10 (3H, s), 5.08 (1H, d, J 4.7Hz), 5.19 (1H, m), 5.83–5.92 (3H, m), 6.32 (2H, br. s, exch), 7.02 (1H, s), 7.63 (1H, br. d, exch., J 8.6Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+(568)].

EXAMPLE 11

Diphenylmethyl (6R,7R)-7-phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) Diphenylmethyl (6R,7R)-7-phenylacetamido-3-(tetrahydrofuran-2-yl)ceph-2-em-4-carboxylate

A solution of (tetrahydrofuran-2-yl)tri-n-butylstannane (J. S. Sawyer, A. Kucerovy, T. L. MacDonald, and G. J. McGarvey, *J. Amer. Chem. Soc.*, 1988, 110, 842) (3.0g, 8.30 mmol) in THF (20ml) was cooled to −78° C. n-Butyl lithium (6.23ml of a 1.6M solution in hexane, 9.97mmol) was then added and the solution was stirred for 15 min. at −78° C. A second flask containing copper (I) bromide.dimethyl sulphide complex (0.854g, 4.14mmol) suspended in a mixture of dimethyl sulphide (15ml) and THF (30ml) was then cooled to −78° C. The α-lithiotetrahydrofuran species was transferred via a cannula to the suspension of copper bromide at −78° C. The red-brown homogeneous solution was stirred for 30 min. at −78° C. A third flask containing a solution of diphenylmethyl 7-phenylacetamido-3-triflyloxyceph-3-em-4-carboxylate (V. Farina, S. R. Baker, and S. I. Hanck, *J. Org. Chem.*, 1989, 54, 4962) (1.9g, 3.0mmol) in a mixture of N-methylpyrrolidinone (20ml) and THF (50ml) was then cooled to −78° C. The cuprate species was transferred via a cannula to the solution of triflate at −78° C. The reaction mixture was stirred for 1h at −78° C. then quenched by the addition of saturated aq. ammonium chloride (30ml). The resulting mixture was allowed to warm to room temperature then diluted with water (100ml) and extracted with ethyl acetate (100ml, 30ml). The combined organic phases were washed with water, brine, then dried over magnesium sulphate. After removal of the solvents under reduced pressure the crude reaction product was purified by flash chromatography on silica gel using 10–30% ethyl acetate/methylene dichloride as eluent. After elution of the 3-n-butylcephem, the title compound was obtained as a mixture of diastereoisomers of the Δ^2 and Δ^3 cephems (1.014g, 61%).

(b) Diphenylmethyl (6R.7R)-1-oxo-7-phenylacetamido-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

A mixture of the cephems (1.014g, 1.83mmol) obtained in Example 11(a) in methylene dichloride (20ml) was cooled to 0° C. A solution of m-chloroperbenzoic acid (0.52g, 60% pure, 1.81mmol) in methylene dichloride (10ml) was then added and the solution was stirred for 10 min. at 0° C. The solution was washed with saturated aq. sodium hydrogen carbonate then water and dried ($MgSO_4$). Evaporation of the solvent gave the title compound (1.005g, 96%) as a mixture of diastereoisomers; ν_{max} (KBr) 1786, 1728 and 1648cm^{-1}; $\delta(CDCl_3)$ 1.41–2.29 (4H, m), 2.99, 3.27 (together 1H, 2d, J 19Hz), 3.63 (2H, br. s), 3.63–3.87 (2.5H, m), 4.20 (0.5H, d), 4.41, 4.43 (together 1H, m), 4.97, 5.14 (together 1H, br. t, J 7.5Hz, and dd, J, 9, 6.9Hz), 6.05, 6.09 (together 1H, 2dd, J 10, 4.7Hz), 6.70, 6.82 (together 1H, 2d, J 10.1Hz), 6.87, 6.94 (together 1H, 2s) and 7.26–7.40 (15H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+ (593)].

(c) Diphenylmethyl (6R,7R)-7-phenylacetamido-3-(tetrahydrofuran-2-yl) ceph-3-em-4-carboxylate

A solution of the sulphoxides (0.975g, 1.71mmol) obtained in Example 11(b) in DMF (20ml) was cooled to −25° C. Phosphorous trichloride (0.30ml, 3.44mmol) was then added and the solution was stirred for 10 min. at −25° C. The reaction mixture was poured onto a mixture of ice, water and ethyl acetate. The organic extract was washed with water, brine, dried ($MgSO_4$) and evaporated. Purification by flash chromatography gave the title compound as a mixture of diastereoisomers (0.811g, 86%); ν_{max} (KBr) 1780, 1723 and 1663cm^{-1}; $\delta(CDCl_3)$ 1.5–2.3 (4H, m), 3.24 (0.5H, d, J 18.6Hz), 3.40 (0.5H, d, J 17.3Hz), 3.56–3.89 (5H, m), −4.84 (0.5H, dd, J 9.1, 6.7Hz), 4.95 (1H, d, J 4.8Hz), 5.01 (0.5H, br t, J 8Hz) 5.76, 5.85 (together 1H, 2dd, J 8.9, 4.8Hz), 6.01, 6.08 (together 1H, 2d, J 8.9Hz), 6.86, 6.94 (1H, 2s) and 7.26–7.38 (15H, m) [mass spectrum: M^+(554)].

EXAMPLE 12

Pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

(a) Pivaloyloxymethyl (6R,7R)-7-phenylacetamido-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

A solution of the cephems (0.811g, 1.46mmol) obtained in Example 11(c) in anisole (5ml) was cooled to 0° C. Trifluoroacetic acid (10ml) was added and the mixture was stirred at 0° C. for 5 min. Toluene was added and the solvents were evaporated off. The residue was partitioned between water and ethyl acetate and the pH was adjusted to 7 by the addition of saturated aq. sodium hydrogen carbonate. The aqueous layer was added to ethyl acetate and the pH taken to 2 by the addition of 1M HCl. The organic phase was washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was dissolved in N-methylpyrrolidinone (3ml). Potassium carbonate (0.426g, 3.08mmol) was added followed by a solution of iodomethyl pivalate (prepared from the bromide 0.438g as in Example 2) in toluene (3ml). The mixture was stirred for 2h at room temperature, then water and ethyl acetate were added. The organic phase was washed

with water, brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography to give the title compound as a (5:1) mixture of diastereoisomers (0.478g, 65%); major diastereoisomer (S) δ_H ($CDCl_3$) 1.22 (9H, s), 1.56 (1H, m), 1.96 (2H, m), 2.35 (1H, m), 3.27 (1H, d, J 18.8Hz), 3.60 (1H, d), 3.65 (2H, ABq, J 16.2Hz), 3.88 (2H, m), 4.86 (1H, dd, J 9.0, 6.7Hz), 4.94 (1H, d, J 4.8Hz), 5.79–6.05 (4H, m) and 7.26–7.38 (5H, m).

(b) Pivaloyloxymethyl (6R,7R)-7-amino-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

A solution of the diastereoisomers obtained in Example 12(a) (0.478g, 0.95mmol) in methylene dichloride (10ml) was cooled to −30° C. N-Methylmorpholine (0.206ml, 1.87mmol) was added followed by a solution of phosphorus pentachloride (0.30g, 1.44mmol) in methylene dichloride (7.5ml). The mixture was stirred at −30° C. for 30 min. Methanol (2.0ml) was added and the mixture was allowed to warm to room temperature over 30 min. Water (2.6ml) was then added and the mixture was stirred vigorously for 1h. The mixture was concentrated by evaporation under reduced pressure and the residue was partitioned between ethyl acetate and water. The pH was adjusted to 7 with 1M aq. ammonia. The organic phase was washed with water, brine, dried ($MgSO_4$) and concentrated. The diastereoisomers were separated by flash chromatography to give (S)-isomer (0.195g); (Found: M^+, 384.1363. $C_{17}H_{24}N_2O_6S$ requires M, 384.1355); ν_{max} (KBr) 3408, 2977, 1780 and 1750cm^{-1}; $\delta(CDCl_3)$, 1.23 (9H, s), 1.64 (1H, m), 1.98 (2H, m), 2.10 (2H, br. s), 2.39 (1H, m), 3.35 (1H, d, J 18.7Hz), 3.63 (1H, d, J 18.6Hz), 3.90 (2H, m), 4.79 (1H, d, J 5.0Hz), 4.88 (1H, dd, J 9.1, 6.7Hz), 4.94 (1H, d, J 5.0Hz) and 5.86 (2H, m). (R)-isomer (0.046mg); $\delta(CDCl_3)$ 1.23 (9H, s), 1.6–2.4 (6H, m), 3.43 (1H, d, J 18Hz), 3.64 (1H, d, J 17.6Hz), 3.88 (2H, m), 4.79 (1H, d, J 4.9Hz), 4.99 (1H, d, J 4.9Hz), 5.17 (1H, t, J 7.5Hz) and 5.87 (2H, m).

(c) Pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S) -tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of (Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid (0.108g, 0.537mmol) in DMF (2ml) was cooled to −50° C. N,N-Diisopropylethylamine (0.103ml, 0.59mmol) followed by methanesulphonyl chloride (0.046ml, 0.59mmol) were added and the mixture was stirred at −50° C. for 30 min. A further quantity of N,N-diisopropylethylamine (0.086ml, 0.493mmol) was added and this mixture was added to a pre-cooled solution of pivaloyloxymethyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (0.185g, 0.482mmol) in DMF (2ml) at 0° C. The resulting mixture was stirred at 0° C. for 40 min., then it was partitioned between ethyl acetate and water. The organic phase was washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography, then triturated with ether to give the title compound (0.193g, 71%) as a white solid. The spectral data was identical with that obtained for Example 8.

(d) Pivaloyloxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of (Z)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid (27mg, 0.134mmol) in DMF (1ml) was cooled to −50° C. N,N-Diisopropylethylamine (26μl, 0.15mmol) followed by methanesulphonyl chloride (11.5μl, 0.15mmol) were added and the mixture was stirred at −50° C. for 30 min. A further quantity of N,N-diisopropylethylamine (22μl, 0.126mmol) was added and this mixture was added to a pre-cooled solution of pivaloyloxymethyl (6R,7R)-7-amino-3-[(R)]-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (46mg, 0.12mmol) in DMF (1ml) at 0° C. The resulting mixture was stirred at 0° C. for 40 min., then it was partitioned between ethyl acetate and water. The organic phase was washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography, then triturated with ether to give the title compound (49.6mg, 73%) as a white solid. The spectral data was identical with that in Example 10.

EXAMPLE 13

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate

(a) (RS)-3-Chloroacetyltetrahydrofuran

(RS)-3-Tetrahydrofuroic acid (3.48g) in dichloromethane (40ml) was treated with oxalyl chloride (11.43g) as described in Example 1(a). The resultant acid chloride in dichloromethane (40ml) was then treated with excess diazomethane (60mM) in ether (100ml), followed by hydrogen chloride. The solution was washed once with brine, dried and concentrated. Flash chromatography on silica gel, eluting with 40% ethyl acetate/hexane afforded the title compound as a pale yellow oil, (3.924g, 88%); ν_{max} (CH_2Cl_2) 1735 and 1716cm^{-1}; 2.17 (2H, dt, J 7.0, 7.5Hz), 3.47–3.58 (1H, m), 3.77–4.04 (4H, m) and 4.18 (2H, s); [mass spectrum: +ve ion (ammonia) MNH_4^+(166)].

(b) (3R,4R)-3-Phenylacetamido-4-[tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-one

(RS)-3-Chloroacetyltetrahydrofuran (0.297g) was coupled with (3R,6R)-4-mercapto-3-phenylacetamidoazetidin-2-one (0.519g) in DMF (4ml), using potassium carbonate (0.304g) as described in Example 1(b). Following work up, the crude product was taken up in hot ethyl acetate and cooled. The crystalline product was filtered off. The solvent was removed from the filtrate and the residue triturated with dichloromethane. The crystalline products were combined to give one diastereoisomer of the title compound, (0.187g, 27%); m.p. 145–155° C. (decomp.); ν_{max} (CH_2Cl_2) 3410, 1748, 1709 (shoulder) and 1688cm^{-1}; δ_H ($(CD_3)_2SO$) 1.74–2.07 (2H, m), 3.26–3.38 (1H, m), 3.48 and 3.56 (2H, ABq, J 16.5Hz), 3.60–3.75 (4H, m), 4.87 (1H, d, J 4.5Hz), 5.24 (1H, dd, J 4.5, 8.4Hz collapses to 1H, d, J 4.5Hz with D_2O) and 9.02 (1H, d, J 8.4Hz, exchangeable with D_2O); [mass spectrum: +ve ion (3NOBA, Na+) MNa^+(371)]. The dichloromethane soluble residue was flash chromatographed with ethyl acetate to give the second diastereoisomer of the title compound as a colourless foam (0.162g, 23%); ν_{max} (CH_2Cl_2) 3407, 3302 (br), 1783 and 1681cm^{-1}; δ_H ($(CD_3)_2SO$) spectrum identical to that of previous isomer except for 3.44–3.58 (2H, m); (mass spectrum: +ve ion (3NOBA, Na^+) MH^+(349), MNa^+ (371)].

(c) t-Butyl (RS)-2-Hydroxy-2-[(3R,4R)-3-phenylacetamido-4-[(RS)-tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate

t-Butyl glyoxylate (1.601g) in 1,2-dichloroethane (20ml) was added to (3R,4R-3-phenylacetamido-4-[(RS)-

tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-one (2.712g) with triethylamine (0.079g, 0.108ml) in 1,2-dichloroethane (5ml) at room temperature; after 1h. the solution was concentrated and flash chromatographed with 70, 80 then 90% ethyl acetate/hexane to give the title compound as a colourless foam, (2.719g, 73%); ν_{max} (CH_2Cl_2) 3415 (br), 1780, 1735, 1685 and 1509cm^{-1}; δ_H ($CDCl_3$) 1.48 and 1.51 (9H, 2s's), 2.03–2.18 (2H, m), 3.20–3.32 (1H, m), 3.46 (1H, d, J 17.5Hz), 3.66 (2H, s), 3.69–3.97 (5H, m), 4.37 and 4.49 (1H, 2 br. d's, J 6.8 and 7.3Hz, exchangeable with D_2O), 4.98 and 5.05 (1H, 2d's, J 4.7 and 4 .6Hz) , 5.15–5.50 (2H, 4m's), 6.43–6.74 (1H, 3m's) and 7.32 (5H, m); [mass spectrum: +ve ion (3NOBA, Na^+) MNa^+(501)].

(d) t-Butyl 2-[(3R,4R)-3-Phenylacetamido-4-[(RS)-tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

t-Butyl (RS)-2-hydroxy-2-[(3R, 4R)-3-phenylacetamido-4-[(RS)-tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate (2.719g) in THF (20ml) was treated with thionyl chloride (1.01g, 0.615ml) and 2,6-lutidine (0.913g, 0.989ml) as described in Example 1(d). Following work-up the crude chloride in dioxan (30ml) was then treated with n-butylphosphine (2.53g, 3.11ml). After purification by flash chromatography with 50, 70% ethyl acetate/hexane then ethyl acetate the title compound was obtained as a pale yellow foam (1.496 g, 40%); ν_{max} (CH_2Cl_2) 3420, 1762, 1717 (shoulder), 1681 and 1625cm^{-1}. (Mass spectrum: +ve ion (3NOBA, Na^+), MH^+(663), MNa^+(685)).

(e) t-Butyl (6R,7R)-7-Phenlacetamido-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate

t-Butyl 2-[(3R, 4R)-3-phenylacetamido-4-[(RS)-tetrahydrofuran-3-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate (1.496g), thermolysed in toluene (30ml) as for Example 1(e) and purified by flash chromatography with 40, 50 and 60 % ethyl acetate/hexane afforded the title compound as a yellow foam (0.28g, 28%); ν_{max} (CH_2Cl_2) 3411, 1702, 1718 and 1687cm^{-1}; δ_H ($CDCl_3$) 1.52 (9H, s), 1.43–2.39 (3H, m's), 3.23 and 3.44 with 3.27 and 3.44 (2H, 2ABq's J 17.7Hz), 3.51–4.03 (6H, m's), 4.94 and 4.96 (1H, 2d's, J 4.7 and 4.7Hz), 5.74–5.82 (1H, m), 6.03 and 6.04 (1H, 2d's, J 8.8 and 8.9Hz) and 7.26–7.42 (5H, m). [Mass spectrum: +ve ion (3NOBA, Na^+) MNa^+(467)].

(f) t-Butyl (6R,7R)-7-Amino-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate

t-Butyl (6R,7R)-7-phenylacetamido-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate (0.9g) in dichloromethane (15ml) with N-methylmorpholine (0.45g, 0.49ml) was successively treated with phosphorus pentachloride (0.549g) in dichloromethane (13.74ml), methanol (10ml) and water (10ml) as described in Example 2(f). After purification by flash chromatography on silica gel eluting with 60, 80% ethyl acetate/hexane and then ethyl acetate, the title compound was obtained as a yellow solid (0.481g, 73%); (Found: M^+, 326.1304. $C_{15}H_{22}N_2O_4S$ requires M, 326.1300); ν_{max} (CH_2Cl_2) 3408, 1775 and 1716cm^{-1}; δ_H ($CDCl_3$) 1.55 (9H, s), 1.69–2.41 (3H, m's), 3.31 and 3.48 with 3.34 and 3.49 (2H, 2ABq's, J 17.5 and 17.5Hz), 3.69–3.83 (4H, 2s's overlapping m), 3.97–4.05 (2H, m), 4.72 and 4.74 (1H, 2d's, J 4.3 and 4.4Hz) and 4.95 and 4.97 (1H, 2d's, J 4.3 and 4.4Hz).

(g) t-Butyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate

2-(Z)-Methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid hydrochloride (0.751g) in DMF (5ml) was treated with methanesulphonyl chloride (0.179g, 0.121ml) and diisopropylethylamine (0.404g, 0.544ml) as described in Example 1(g). This was then treated with t-butyl (6R,7R)-7-amino-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate (0.464g) and pyridine (0.112g, 0.114ml) in DMF (5ml). Following work up and purification by flash chromatography with 40, 50 and 60% ethyl acetate/hexane, the title compound was obtained as a yellow foam (0.874g, 82%); ν_{max} (CH_2Cl_2) 3398, 1783, 1731 (shoulder), 1718 and 1688cm^{-1}; δ_H ($CDCl_3$) 1.53 (9H, s), 1.69–2.43 (3H, m's), 3.29 and 3.46 with 3.34 and 3.48 (2H, 2ABq's, J 17.7 and 17.7Hz), 3.63–4.07 (6H, m's and s), 5.03 and 5.06 (1H, 2d's, J 4.8 and 4.8Hz), 5.84–5.90 (1H, m), 6.73 and 6.74 (1H, 2s), 6.76 and 6.90 (1H, 2d's, J 8.7 and 8.7Hz exchangeable with D_2O), 7.02 (1H, br. s, exchangeable with D_2O) and 7.31 (15H, s). [Mass spectrum: +ve ion (3NOBA, Na^+) MNa^+ (774)].

(h) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido-3-(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate

t-Butyl (6R,7R)-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate (0.859g) was deprotected in 10% 1M hydrochloric acid in formic acid (11.4ml) as described in Example 1(h). After work up the pH of the solution was adjusted to 8 with aqueous sodium hydrogen carbonate, and the product purified by column chromatography on HP20SS eluting with 1, 2, 4 and 6% THF/water. The fractions containing the product by h.p.l.c., were combined, concentrated and freeze-dried to give the title compound as an amorphous white solid (0.4g, 74%); ν_{max} (KBr) 1757, 1670, 1596 and 1532cm^{-1}; δ_H ($(CD_3)_2SO$) 1.61–2.08 (3H, m's), 3.15 and 3.37 with 3.18 and 3.37 (2H, 2ABq's, J 16.6 and 16.7Hz), 3.45–3.66 (2H, m), 3.76–3.95 (5H, m overlapping s at 3.84), 4.96 and 4.97 (1H, 2d's, J 4.3 and 4.6Hz), 5.46–5.54 (1H, m), 6.75 and 6.76 (1H, 2s's), 7.25 (2H, br s, exchangeable with D_2O). [Mass spectrum: +ve ion (thioglycerol) MH^+(476), MNa^+(498)].

EXAMPLE 14

4-Methoxybenzyl (6R,7R)-7-Amino-3-((R)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

(a) (R)-2-Bromoacetyltetrahydrofuran

(R)-2-Tetrahydrofuroic acid (2.739g, EPA 0382 506) was converted to it's acid chloride with oxalyl chloride (9g, 6.18ml) as previously described in Example 1(a). This was dissolved in dichloromethane, cooled in ice/water and saturated with excess diazomethane, bubbled through the solution in a stream of argon. 48% Aqueous hydrogen bromide (4.41ml) was then added and the reaction mixture vigorously stirred. After 10 min. the solution was washed with brine, dried and concentrated. Flash chromatography eluting with 5% then 10% ethyl acetate/hexane afforded the title compound as a pale yellow oil (3.519g, 77%); $[\alpha]_D$+60.9 (C 1.01 $CHCl_3$).

(b) 4-Methoxybenzyl (RS)-2-Hydroxy-2-[(3R,4R)-3-phenylacetamido-4-(R)-tetrahydrofuran-2-ylcarbonylmethylthio)-azetidin-2-on-1-yl]acetate

4-Methoxybenzyl (RS)-2-hydroxy-2-[(1R,5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl]acetate (4.103g) in dichloromethane (15ml) and acetone (15ml) was ring opened with 4-toluenesulphonic acid hydrate (3.33g) in

water (8ml) and coupled to (R)-2-bromoacetyltetrahydrofuran (2.11g) in acetone (20ml) with potassium carbonate (0.687g) as described in Example 6(b) for the diastereoisomeric mixture. After purification by flash chromatography, the title compound was obtained as a yellow gum (2.618g, 49%); $[\alpha]_D$–10.7 (c 1.00 $CHCl_3$).

(c) 4-Methoxybenzyl 2-[(3R,4R)-3-Phenylacetamido-4-[(R)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

4-Methoxybenzyl (RS)-2-hydroxy-2-[(3R,4R)-3-phenylacetamido-4-((R)-tetrahydrofuran-2-ylcarbonylmethylthio)azetidin-2-on-1-ylacetate (2.558g) was converted to the title compound with thionyl chloride (0.842g, 0.51ml), 2,6-lutidine (0.757g, 0.82ml) and tri-n-butylphosphine (2.1g, 2.58ml) as described for the diastereoisomeric mixture in Example 6(b). The product was obtained as a brown gum (2.16g, 63%).

(d) 4-Methoxybenzyl (6R,7R)-7-Phenylacetamido-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The phosphorane (2.16g) prepared in Example 14(c), in toluene (50ml) was heated under reflux for 8h. Removal of solvent and chromatography afforded the title compound as a yellow solid (1.008g, 67%).

(e) 4-Methoxybenzyl (6R,7R)-7-Amino-3-((R)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

The cephem (0.98g), prepared in Example 14(d) was treated with phosphorus pentachloride (0.523g) in dichloromethane (13.1ml) and N-methylmorpholine (0.429g, 0.466ml), then methanol (10mls) and water (10ml) as described for the diastereoisomeric mixture in Example 6(e). After work up and purification by crystallisation from toluene, the title compound was obtained as a colourless solid (0.252g, 33%); m.p. 130–132° C.; $[\alpha]_D$+11.5 (c 1.00 $CHCl_3$); ^1H n.m.r. was shown to be identical to that obtained for (R)-isomer prepared in Example 6(e).

EXAMPLE 15

4-Methoxybenzyl (6R,7R)-7-Amino-3-((S)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

(a) (S)-2-Bromoacetyltetrahydrofuran

(S)-2-Tetrahydrofuroic acid (5.94g) was converted to it's acid chloride with oxalyl chloride (13.00g). This was then converted to the title compound with diazomethane and then 48% aqueous hydrogen bromide (9.58ml) as described in Example 14(a). After isolation, the product was obtained as pale yellow oil (8.78g, 89%); $[\alpha]_D$–62.8 (c 1.00, $CHCl_3$).

(b) 4-Methoxybenzyl (RS)-2-Hydroxy-2-[(3R,4R)-3-phenylacetamido-4-((S)-tetrahydrofuran-2-ylcarbonylmethylthio)-azetidin-2-on-1-yl]acetate

4-Methoxybenzyl (RS)-2-hydroxy-2-[(1R,5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl]acetate (15.09g) in 50% acetone/dichloromethane (100ml) was cleaved with 4-toluenesulphonic acid (12.25g) in water (25ml). This product was reacted with the crude bromide from Example 15(a) (8.78g) in acetone (40ml) in the presence of potassium carbonate (2.53g) as described in Example 14(b). The title compound was obtained as a yellow foam (12.366g, 62%).

(c) 4-Methoxybenzyl 2-[(3R,4R)-3-Phenylacetamido-4-[(S)-tetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

As for Example 14(c) the alcohol from 15(b) (12.366g) was converted to the title compound with thionyl chloride (2.47ml) and 2,6-lutidine (3.99ml) followed by tri-n-butylphosphine (12.55ml). After purification the phosphorane was obtained as a brown gum (10g, 60%).

(d) 4-Methoxybenzyl (6R,7R)-7-Phenylacetamido-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

As for Example 14(d) the phosphorane from Example 15(c), (10g) was cyclized in refluxing toluene (200mls). After isolation, the title compound was obtained as a yellow foam (5.452g, 78%).

(e) 4-Methoxybenzyl (6R,7R)-7-Amino-3-((S)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

As for Example 14(e) the cephem from 15(d), (5.452g) was treated with phosphorus pentachloride (2.96g) and N-methylmorpholine (2.9ml) in dichloromethane (125ml), followed by treatment with methanol (50ml) then water (50ml). After adjusting the pH to 7 with 0.880 ammonium hydroxide and purification, the title compound was obtained as a pale yellow foam (2.803g, 67%); ^1H n.m.r. was shown to be identical to that obtained for the S-isomer prepared in Example 6(c).

EXAMPLE 16

Acetoxymethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) Acetoxymethyl (6R,7R)-7-phenylacetamido-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

To a solution of (6R,7R)-7-phenylacetamido-3-(tetrahydrofuran-2-yl)ceph-3-em-4-carboxylic acid (0.303g, 0.78mmol) (obtained in Example 12) in N-methylpyrrolidinone (5ml) was added potassium carbonate (0.37g, 2.66mmol). Bromomethyl acetate (0.30g, 1.95mmol) was added dropwise to the mixture over 1h. The mixture was stirred for a further 1h, then ethyl acetate and water were added. The organic phase was washed with water, brine, dried ($MgSO_4$) and evaporated. The residue was purified by chromatography to give the title compound as a mixture of diastereomers (0.198g, 56%); major diastereomer(S); δ_H ($CDCl_3$) 1.59 (1H, m), 1.95 (2H, m), 2.12 (3H, s), 2.38 (1H, m), 3.28 (1H, d, J 18.9Hz), 3.59 (1H, d), 3.65 (2H, ABq, J 16.4Hz), 3.88 (2H, m), 4.89 (1H, dd, J 9.0, 6.7Hz), 4.93 (1H, d, J 4.9Hz), 5.84 (3H, m), 6.01 (1H, d, 9.1Hz) and 7.34 (5H, m).

(b) Acetoxymethyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

As for Example 12(b), the cephem from Example 16(a), (0.196g) was treated with phosphorus pentachloride (132mg) and N-methylmorpholine (94μl) in dichloromethane (7ml), followed by treatment with methanol (0.85ml) then water (1.15ml). After adjusting the pH to 7 with 1M aq. ammonia and work up, the diastereomers were separated by flash chromatography to give the (S)-isomer (54.3mg, 37%); δ_H ($CDCl_3$) 1.66 (1H, m), 1.97 (2H, m), 2.13 (3H, s), 2.40 (1H, m), 3.56 (2H, ABq, J 17.6Hz), 3.91 (2H, m), 5.03 (3H, m) and 5.84 (2H, m).

(c) Acetoxymethyl (6R,7R)-7-[(Z)-2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylate

As for Example 12(c), 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (35mg) was treated with N,N-diisopropylethylamine (34 and 27μl) and methanesulphonyl chloride (15μl) in DMF (1ml)and then added to the amino compound (53mg) obtained in Example 16(b) in DMF (1ml). After work up and chromatography the title compound (60mg, 74%) was obtained as a foam; ν_{max} (KBr) 3330, 1774, and 1676cm^{-1}; δ_H ($CDCl_3$) 1.64 (1H, m), 1.99 (2H, m), 2.14 (3H, s), 2.41 (1H, m), 3.38 and 3.67 (2H, ABq, J 18.9Hz), 3.90 (2H, m), 4.11 (3H, s), 4.95 (1H, dd, J 9.0, 6.8Hz), 5.07 (1H, d, J 4.8Hz), 5.86 (2H, m), 5.99 (1H, dd, J 8.9, 4.8Hz), 6.08 (2H, brs), 7.00 (1H, s) and 7.49 (1H, d, J 8.8Hz). [Mass spectrum: +ve ion (ammonia) 526 (MH^+)].

EXAMPLE 17

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5-methoxymethyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

(a) (2RS,5SR)-5-Methoxymethyltetrahydrofuran-2-carboxylic acid

A solution of 5-methoxymethylfuran-2-carboxylic acid (3.10g) in ethyl acetate (40ml) was hydrogenated over 5% rhodium on carbon (200mg) until hydrogen uptake ceased. The catalyst was filtered off and washed with ethyl acetate. The combined filtrates were evaporated to give the title compound as a colourless oil (3.26g); ν_{max} (film) 1750cm^{-1}; δH ($CDCl_3$) 1.75–2.1 (2H,m), 2.1–2.6(2H,m,), 3.45 (3H,s), 3.47 (1H,dd, J 3 and 10 Hz), 3.74 (1H,dd, J 4 and 10Hz), 4.15–4.43 (1H,m) and 4.43–4.63 (1H,m).

(b) (2RS,5SR)-2-Chloroacetyl-5-methoxymethyltetrahydrofuran

A solution of (2RS,5SR)-5-methoxymethyltetrahydrofuran-2-carboxylic acid (3.1g) in dichloromethane (50ml) was treated with oxalyl chloride (2.68ml) and dimethylformamide (1 drop). The mixture was stirred for 1h and heated to reflux for 10 mins. The solvent was evaporated and then dichloromethane was evaporated from the residue twice. The product was dissolved in dichloromethane (100ml) and the solution cooled in an ice bath. Diazomethane was then passed into the solution as described in Example 14(a). When the addition was complete the mixture was stirred at 0° C. for 0.5h and then hydrogen chloride gas was passed into the solution until all the diazoketone had been consumed. The solution was washed with brine, dried over magnesium sulphate and evaporated. The title compound (2.44g) was isolated by column chromatography using gradient elution (silica gel, 4:1 going to 1:1 hexane : ethyl acetate); ν_{max} (film) 1740cm^{-1}; δ_H ($CDCl_3$) 1.6–2.35 (4H,m), 3.30–3.75 (2H,m), 3.37 (3H,s) and 4.05–4.75 (4H,m).

(c) (3R,4R)-4-[(2RS,5SR)-5-Methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenoxyacetamidoazetidin-2-one

Potassium carbonate (1.0g) was added to a stirred mixture of (3R,4R)-4-mercapto-3-phenoxyacetamidoazetidin-2-one (1.07g) and (2RS,5SR) 2-chloroacetyl-5-methoxymethyltetrahydrofuran (0.869g) in dimethylformamide (15ml). The mixture was stirred at room temperature for 1.5h and then partitioned between ethyl acetate and water. The organic phase was washed twice with water, then brine, dried over magnesium sulphate and evaporated. The product (0.987g) was isolated by column chromatography of the residue (silica gel, ethyl acetate as eluent); ν_{max} ($CHCl_3$) 3411, 3308, 1779 and 1689 cm^{-1}; δ_H ($CDCl_3$) 1.63–1.77 (1H,m), 1.88–2.23 (3H,m), 3.30–3.62 (6H,m), 3.65–3.78 (1H,m), 4.15–4.30, (1H,m), 4.42–4.51 (1H,m), 4.57 (2H,s), 5.04 (1H, d, J 4.0Hz)., 5.60 (1H, dd, J 4.35, 9.09Hz) 6.90–7.08 (4H,m), 7.28–7.49 (2H, m) and 7.49 (1H,t, J 8.16Hz).

(d) 4-Methoxybenzyl (2RS)-2-Hydroxy-2-[(3R,4R)-4-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenoxyacetamidoazetidin-2-on-1-yl]-acetate

A solution of 4-methoxybenzyl glyoxylate (1.82g) in dichloroethane (30ml) was heated at reflux using a Dean and Stark water separator for 1h. The solution was then cooled to room temperature and then (3R,4R)-4-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenoxyacetamidoazetidin-2-one (2.94g) in dichloroethane (20ml) was added followed by triethylamine (0.1ml). The mixture was stirred at room temperature for 1h and then the solvents were removed on a rotary evaporator. The product was isolated as a mixture of isomers (3.23g) by column chromatography of the residue (silica gel, ethyl acetate as eluent); ν_{max} ($CHCl_3$) 3411, 1780, 1745 and 1691cm^{-1}.

(e) 4-Methoxybenzyl 2-[(3R,4R)-4-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio)]-3-phenoxyacetamidoazetidin-2-on-1-yl]-2-tri-n-butyl-phosphoranylideneacetate.

2,6-Lutidine (0.95ml) was added to a stirred solution of 4-methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenoxyacetamidoazetidin-2-on-1-yl]acetate in tetrahydrofuran (24ml). A solution of thionyl chloride (0.59ml) in tetrahydrofuran (4ml) was then added at <–20° C. and the mixture was stirred for 2h. The solution was filtered and evaporated and the residue was dissolved in toluene and evaporated again. The crude product was dissolved in dioxan under argon and tri-n-butylphosphine (3.0ml) was added. The mixture was stirred at room temperature for 0.5h and then diluted with ethyl acetate and washed with sodium bicarbonate solution, water and brine. The solution was dried over magnesium sulphate and evaporated. The title compound (4.25g) was isolated by column chromatography of the residue using gradient elution (silica gel 1:1 hexane : ethyl acetate going to neat ethyl acetate); ν_{max} ($CHCl_3$) 3421, 1761, 1688 and 1612cm^{-1}.

(f) 4-Methoxybenzyl (6R,7R)-3-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-yl]-7-phenoxyacetamidoceph-3-em-4-carboxylate.

A solution of 4-methoxybenzyl 2-[(3R,4R)-4-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenoxyacetamidoazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate (4.25g) and benzoic acid (20mg) in toluene (100ml) was heated to reflux for 10h. The mixture was cooled and the solvent evaporated. The product (1.93g) was isolated by column chromatography of the residue using gradient elution (silica gel 1:1

hexane : ethyl acetate going to neat ethyl acetate); ν_{max} ($CHCl_3$) 3409, 1784, 1722 and 1695cm^{-1}.

(g) 4-Methoxybenzyl (6R,7R)-7-Amino-3-(5-methoxymethyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate.

A solution of 4-methoxybenzyl (6R,7R)-3-[(2RS,5SR)-5-methoxymethyltetrahydrofuran-2-yl]-7-phenoxyacetamidoceph-3-em-4-carboxylate (1.93g) in dichloromethane (25ml) was cooled to −15 to −20° C., N-methylmorpholine (0.75ml) was added followed by a solution of phosphorus pentachloride in dichloromethane (26.5ml of a solution containing 40mg.ml^{-1}). The mixture was stirred at the same temperature for 0.5h and then methanol (6.8ml) was added and the mixture stirred at room temperature for 0.5h. Water (10ml) was then added and the mixture vigorously stirred for 0.5h. The dichloromethane was then removed on a rotary evaporator and the residue was partitioned between ether and water. The aqueous phase was stirred with ethyl acetate and the pH was adjusted to 6.2 with 1N aqueous ammonia. The organic phase was washed with water and brine, dried over magnesium sulphate and evaporated. The products were separated by column chromatography of the residue using gradient elution is (silica gel, 1:1 hexane : ethyl acetate going to neat ethyl acetate). The following were obtained in order of elution 4-methoxybenzyl (6R,7R)-7-amino-3-[(2S,5R)-5-methoxymethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (388mg); ν_{max} ($CHCl_3$) 3410, 1776 and 1725cm^{-1}; δ_H ($CDCl_3$) 1.59–1.78 (2H,m), 1.93–2.08 (1H, m), 2.18–2.32 (1H,m), 2.54 (2H, br s), 3.33–3.54 (3H,m), 3.38 (3H,s), 3.80 (3H,s), 4.00–4.11 (1H,m), 4.76 (1H,d, J 4.99Hz), 4.90 (1H,d, J 4.97Hz) 4.96 (1H,t, J 8.23Hz) 5.17 (2H,s), 6.88 (2H,d, J 8.60Hz) and 7.33 (2H,d, J 8.61Hz). [Mass spectrum: M$^+$(434)]; 4-methoxybenzyl (6R,7R)-7-amino-3-[(2R,5S)-5-methoxymethyltetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (305mg); ν_{max} ($CHCl_3$) 3409, 1776 and 1725cm^{-1}; δ_H($CDCl_3$) 1.60–1.81 (2H, m), 1.85–2.01 (2H, m), 3.30–3.50 (2H, m), 3.38 (3H, s), 3.44 (1H,d, J 17.78Hz), 3.69 (1H, d, J 17.75Hz), 3.80 (3H, s), 4.00–4.17 (1H,m), 4.70 (1H, d, J 4.92Hz), 4.93 (1H, d, J 4.95Hz), 5.10–5.20 (1H, m), 5.18 (1H, d, J 11.88Hz), 5.24 (1H, d, J 11.89Hz), 6.88 (1H,d, J 8.65Hz) and 7.35 (1H, d, J 8.64Hz). [Mass spectrum: M+ (434)].

(h) 4-Methoxybenzyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2R,5S)-5-methoxymethyltetrahydrofuran-2-yl] ceph-3-em-4-carboxylate

A stirred solution of 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (155mg) and N,N-diisopropylethylamine (134μl) in dimethylformamide (3ml) was cooled to −30° to −40° C. and methanesulphonyl chloride (60μl) was added. The mixture was stirred at the same temperature for 0.5h and then a solution of 4-methoxybenzyl (6R,7R)-7-amino-[(2R,5S)-5-methoxymethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (304mg) in dimethylformamide (3ml) was added followed by pyridine (60μl). The mixture was then stirred at 0° C. for 1.5h, and then partitioned between ethyl acetate and aqueous citric acid solution. The organic phase was washed three times with water, then with brine, dried over magnesium sulphate and evaporated. The title compound (115mg) was isolated by column chromatography of the residue using gradient elution (silica gel 1:1 hexane : ethyl acetate going to neat ethyl acetate), ν_{max} ($CHCl_3$) 3489, 3397, 3330, 1779, 1723, 1681cm^{-1}; δ_H ($CDCl_3$) 1.60–1.80 (2H,m), 1.78–2.05 (2H,m), 3.30–3.53 (3H,m), 3.37 (3H,s), 3.70 (1H,d, J 17.87Hz), 3.81 (3H,s), 4.08 (3H,s), 5.05 (1H,d, J 4.79Hz), 5.18 (1H,d, J 11.81Hz), 5.24 (1H,d, J 11.62Hz), 5.90 (1H,dd, J 4.75 and 8.89Hz), 6.90 (1H,d, J 9.56Hz), 6.91 (1H,s), 7.34 (1H,d, J 8.67Hz) and 7.67 (1H,d, J 8.88Hz).

(i) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2SR,5S)-5-methoxymethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

Concentrated hydrochloric acid (0.15ml) was added to a stirred solution of 4-methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2R, 5S)-5-methoxymethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (115mg) in 95% formic acid (4ml). The mixture was stirred at room temperature for 1.5h and then the solvents were removed on a rotary evaporator, and then toluene was evaporated from the residue twice. The residue was stirred with water and toluene and the pH of the aqueous phase was adjusted to 6.2 with aqueous sodium bicarbonate solution. The aqueous phase was separated and evaporated and the title compound (36mg) was obtained as a mixture of isomers by column chromatography of the residue (HP20SS water with increasing proportions of acetone as eluent). Fractions containing product were combined, evaporated, and the residue dissolved in water (5ml) and freeze-dried; ν_{max} (KBr) 1762, 1671 and 1602 cm^{-1}; δ_H [$(CD_3)_2SO$] 1.4–2.15 (4H,m), 3.14–3.48 (4H,m), 3.24 and 3.27 (3H, 2s), 3.83 (3H,s), 3.87–3.98 and 4.03–4.18 (1H,m), 4.96 (1H,d,J 4.66Hz), 5.00 and 5.22 (1H,2t, J 7.47Hz), 5.46–5.57 (1H,m), 6.74 and 6.75 (1H,2s), 7.25 (2H,s) and 9.49 and 9.53 (1H, 2d, J 8.12Hz)

EXAMPLE 18

Sodium (6R,7R)-7-[(2-(2-Aminothiazol-4-yl)-(Z)-pent-2-enamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-(Z)-pent-2-enamido-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

Mesyl chloride (70μl) was added to 2-(2-aminothiazol-4-yl)-(Z)-pent-2-enoic acid (178mg) and N,N-diisopropylethylamine (160μl) in DMF (5ml) and dichloromethane (5ml) at −20° C. The reaction mixture was stirred at −20° C. for 1 hour then added to an icecold solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (370mg) and N,N-diisopropylethylamine (160μl) in dichloromethane (5ml). Stirred for 1 hour, concentrated and flash chromatographed on silica gel eluting with 30, 50, 60 and 70% ethyl acetate in hexane to give the title compound (90mg); ν_{max} ($CHCl_3$) 1782, 1720, 1674, 1614, 1516, 1134 and 1107cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.12 (3H,t,J 7.5Hz) 1.50–2.45 (6H,m), 3.30–3.95 (7H,m), 4.85–5.05(2H,m), 5.18 (2H,s), 5.85–5.95 (1H,m), 6.44 (1H,s), 6.52 (1H,t,J 7.8Hz), 6.90 and 7.32 (4H, ABq, J 8.6Hz) and 7.43 (1H,d, J 8Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa$^+$(689)].

(b) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-(Z)-pent-2-enamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate

4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-pent-2-enamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-

em-4-carboxylate (80mg) in dichloromethane (2ml) was added dropwise to a mixture of aluminium chloride (47mg) and anisole (1.03ml) in dichloromethane (2ml) at −50° C. under argon. The mixture was stirred for 15 minutes at −40° C. and 0.5M trisodium citrate (3.42ml) added, stirred at room temperature for 15 minutes then diluted with dichloromethane (10ml) and water (10ml). The aqueous layer was collected, washed with dichloromethane and chromatographed on HP20SS eluting with 0, 1, 2, 5 and 10% acetone in water. Fractions containing the product, h.p.l.c analysis, were combined, concentrated and freeze-dried to give the title compound (22mg); ν_{max} (KBr) 3407, 1757, 1609, 1527, 1375, 1338 and 1041 cm^{-1}; δ_H (D_2O, 250MHz) 1.03 (3H,t,J 7.5Hz), 1.65–2.30 (6H,m), 3.32 and 3.51 (2H, ABq, J 7.7Hz), 3.70–3.95 (2H,m), 4.65–4.80 (1H,m), 5.20 (1H,d,J 4.7Hz), 5.74 (1H,d,J 4.7Hz), 6.33 (1H,t,J 8Hz) and 6.47 (1H,s). [Mass spectrum +ve ion (thioglycerol) MH^+(473)].

EXAMPLE 19

Sodium (6R,7R)-7-[2-(2-Aminothiadiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-tritylaminothiadiazol-4-yl) acetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

Mesyl chloride (65μl) was added to 2-(Z)-methoxyimino-2-(2-tritylaminothiadiazol-4-yl)acetic acid (370mg) and N,N-diisopropylethylamine (146μl) in dichloromethane (5ml) at −20° C. The reaction mixture was stirred at −20° C. for 1 hour then added to an ice cold solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (335mg) and pyridine (70μl) in dichloromethane (5ml). The reaction was stirred for 1 hour, concentrated and flash chromatographed on silica gel eluting with 30,50,60 and 70% ethyl acetate in hexane to afford the title compound as a foam (300mg); ν_{max} ($CHCl_3$) 3398, 1784, 1724, 1691, 1516, 1134 and 1107 cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.55–1.75 (1H,m), 1.80–2.05 (2H,m), 2.25–2.45 (1H,m), 3.30 and 3.61 (2H, ABq, J 18.3Hz), 3.75–4.00 (2H,m), 3.81 (3H,m), 4.16 (3H,m), 4.85–5.00 (1H,m), 5.00 (1H,d, J 4.8Hz), 5.17 (2H,s), 5.92 (1H,dd, J 4.8Hz), 6.72 (1H,d,J 7.8Hz) and 6.88 and 7.30 (19H,m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(839)].

(b) Sodium (6R,7R,)-7-[2-(2-Aminothiadiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

Trifluoroacetic acid (5ml) was added to 4-methoxybenzyl (6R,7R)-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiadiazol-4-yl)acetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (100mg) and anisole (1ml) in dichloromethane (5ml) at room temperature and stirred for 1 hour. The mixture was evaporated and re-evaporated from toluene (10ml). The residue was dissolved in 1% sodium hydrogen carbonate solution (1ml), washed with ether and chromatographed on HP20SS eluting with 0,0.5 and 1% acetone in water. Fractions containing the product, h.p.l.c analysis, were combined, concentrated and freeze dried to give the title compound (35mg); ν_{max} (KBr) 3381, 1758, 1669, 1602, 1527, 1399 and 1042cm^{-1}; δ_H (D_2O) 1.64–2.25 (4H,m), 3.30 and 3.49 (2H, ABq, J 17.8Hz), 3.70–3.95 (2H,m), 4.03 (3H,s), 4.65–4.75 (1H,m), 5.19 (1H,d,J 4.7Hz) and 5.77 (1H,d,J 4.7Hz). [Mass spectrum: +ve ion (thioglycerol) MH^+(477)].

EXAMPLE 20

(RS)-1-Acetoxyethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

A solution of (RS)-1-acetoxyethylbromide (267mg) in 1-methyl-2-pyrrolidinone (2ml) was added dropwise, over 1 hour, to an ice cold mixture of sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (190mg) and potassium carbonate (110mg) in 1-methyl-2-pyrrolidinone (1ml). After 15 minutes the mixture was diluted with ethyl acetate, washed with water, brine, dried ($MgSO_4$), concentrated and flash chromatographed on silica gel eluting with 50,70,80 and 90% ethyl acetate in hexane to give the title compound (172mg); ν_{max} ($CHCl_3$) 3019, 2929, 1786, 1683, 1520, 1376 and 1135cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.45–1.75 (4H,m), 1.90–2.10 (2H,m), 2.09 and 2.10 (together 3H, 2s), 2.30–2.50 (1H,m), 3.36 and 3.65 (2H, ABq, J 18.8Hz), 4.93–5.10 (2H,m), 5.90–6.05 (1H,m), 6.94 and 7.07 (together 1H,q,J 5.8Hz), 7.10 and 7.15 (together 1H, 2s) and 7.60 and 7.67 (together 1H,2d, J 7.4Hz); [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+ (562)].

EXAMPLE 21

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-carboxymethoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylic acid disodium salt.

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(Z)-t-butoxycarbonylmethoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

2-[(2)-t-Butyloxycarbonylmethoxyimino]-2-(2-tritylaminothiazol-4-yl)acetic acid (179mg, 0.31mmol) in DMF (4ml) was treated at −25° C. with N,N-diisopropylethylamine (52μl, 0.31mmol) and methanesulphonyl chloride (24μl, 0.31mmol) for 30 min. A mixture of 4-methoxybenzyl (6R,7R)-7-amino-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (119mg, 0.31 mmol) [See example 6] and pyridine (26μl, 0.31mmol) dissolved in DMF (4ml) was added and stirring was maintained at 0° C. for 1h. The reaction mixture was partitioned between ethyl acetate and dilute aqueous sodium hydrogen carbonate, the organic layer was washed with aqueous citric acid then water, dried (magnesium sulphate) and evaporated to low bulk. The residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give the title compound as a cream amorphous solid (190mg, 69%); δ_H ($CDCl_3$) 1.43 (9H,s), 1.54–1.68 (2H,m), 1.86–1.95 (1H,m), 2.02–2.12 (1H,m), 3.34 and 3.50 (2H, ABq, J 18Hz), 3.76–3.91 (2H,m), 3.81 (3H,s), 4.76 (2H, br s), 5.02 (1H, d, J 5Hz), 5.16–5.22 (1H,m), 5.78 (1H, dd, J 5,8Hz), 6.84 (1H,s), 6.8 (2H, d, J 9Hz), 7.0 (1H, br s, exch) and 7.26–7.36 (17H,m). [Mass spectrum: +ve ion (3-nitrobenzylalcohol, sodium acetate) MNa^+(938)].

(b) (6R,7R)-7-[2-Aminothiazol-4-yl)-2-(Z)-carboxymethoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylic acid disodium salt.

The product of Example 21(a) (174mg, 0.19m mol) was dissolved in a mixture of trifluoroacetic acid; dichlo-

romethane and anisole (4:4:1, 5ml) and kept at room temperature for 2h. The solution was evaporated to dryness under reduced pressure and the residue was twice washed with ether. The residue solid was dissolved in water using sodium hydrogen carbonate to bring to pH7.5 then the solution was chromatographed on HP20SS eluting with water. There was some separation of isomers but most of the product was collected as a mixed fraction of (R) and (S) tetrahydrofuryl isomers which was freeze dried to a white solid (42mg, 44%), ν_{max} (KBr) 1761, 1660, (sh) 1601 and 1533 cm^{-1}; δ_H (D_2O) (major isomer) 1.69–2.18 (4H,m), 3.32 and 3.51 (2H, ABq, J 18Hz), 3.74–3.93 (2H,m), 4.52 (2H,s), 5.19 (1H, d, J 5Hz), 5.77 (1H, d, J 5Hz) and 7.01 (1H,s); (minor isomer) (inter alia), 3.37 and 3.57 (ABq, J 17Hz), 5.17 (d, J 5Hz) and 5.76 (d, J 5Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+ (563)].

EXAMPLE 22

Sodium (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

(a) 4-Methoxybenzyl (6R,7R)-7-[(R)-2-t-butoxycarbonylamino-2-(4-hydroxyphenyl) acetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

4-Methoxybenzyl (6R,7R)-7-amino-3-[(R)-tetrahydrofuran-2-yl)ceph-3-em-4-carboxylate (136mg, 0.35mmol) [See example 6] in THF (10ml) was stirred in an ice bath with dicyclohexylcarbodiimide (108mg, 0.52mmol) then (R)-2-t-butoxycarbonylamino-2-(4-hydroxyphenyl) acetic acid (139mg, 0.52mmol) in THF (3ml) was added dropwise over 2 min. The mixture was stirred at 0° C. for 30 min then at room temperature for 30 min. It was filtered and evaporated and the residue chromatographed on silica gel eluting with ethyl acetate/hexane mixtures. The title compound was obtained as a white solid (212mg, 95%); δ_H ($CDCl_3$) 1.10–2.0 (4H,m), 1.42 (9H,s), 3.18 and 3.43 (2H, ABq, J 17Hz), 3.80 (3H,s), 3.77–3.88 (2H,m), 4.89 (1H,d, J 5Hz), 5.10 (1H,t, J 7Hz), 5.11 (1H,d, J 5Hz), 5.19 (2H, s), 5.65 (1H,d, J 5Hz exch), 5.69 (1H,dd, J 4,9Hz), 6.72 (2H,d, J 8Hz), 6.81 (1H,d, J 9Hz exch), 6.88 (2H,d, J 9Hz), 7.10 (2H,d, J 8Hz) and 7.33 (2H,d,J 9Hz). (Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(938)].

(b) Sodium (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)-acetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The product of Example 22(a) (42mg, 0.66mmol) was treated as in Example 21(b). The final chromatography on HP20SS yielded two fractions. The first fraction to be eluted was the pure (S)-tetrahydrofuran-2-yl isomer (53mg, 19%) as a white freeze dried solid; ν_{max} (KBr) 1762, 1690 and 1600cm^{-1}; δ_H (D_2O) 1.62–1.74 (1H,m), 1.87–1.98 (2H,m), 2.15–2.05 (1H,m), 3.10 and 3.39 (2H, ABq, J 18Hz), 3.72–3.90 (2H,m), 4.66 (1H,t, J 8Hz), 5.04 (1H,d, J 4.5Hz), 5.61 (1H,d, J 4.5Hz), 6.90 (1H,d, J 9HZ) and 7.31 (2H,d, J 9Hz). Further elution of the column gave a mixture of diastereoisomers (84mg, 30%).

EXAMPLE 23

Sodium (1S,6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1-oxide

(a) 4-Methoxybenzyl (1S,6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1-oxide

4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (see example 7) (250mg, 0.44mmol) in ethyl acetate (25ml) was stirred in an ice bath and a solution of m-chloroperbenzoic acid (75mg, 0.44mmol) in ethyl acetate (5ml) was added. After 10 min the reaction mixture was washed with dilute aqueous sodium hydrogen carbonate then water followed by drying (magnesium sulphate) and evaporation under reduced pressure. The residue was chromatographed on silica gel eluting with acetone/ethyl acetate mixtures to give the title compound as a white solid (179mg, 69%); ν_{max} ($CHCl_3$) 1800, 1730, 1680 and 1610 cm^{-1}; δ_H ($CDCl_3$) 1.48–1.64 (1H,m), 1.89–2.00 (2H,m), 2.33–2.47 (1H,m), 3.29 and 3.75 (2H, ABq, J 19Hz), 3.82 (3H,s), 3.84–3.96 (2H,m), 4.09 (3H,s), 5.06 (1H,dd, J 7, 9Hz), 5.22 (2H,s), 5.55–5.8 (1H, br s, exch), 6.16 (1H,dd, J 4.5, 10Hz), 6.91 (2H,d, J 7,9Hz), 6.98 (1H,s), 7.35 (2H,d, J 9Hz) and 7.55–7.65 (1H,br, exch). [Mass spectrum: +ve ion (thioglycerol) MH^+(590)].

(b) Sodium (1S,6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1-oxide

Anhydrous aluminium chloride (115mg, 0.86mmol) was added to a mixture of anisole (5ml) and dichloromethane (3ml) cooled to –20° C. After 15 mins at –20° C. the mixture was cooled to –40° C. and a solution of the product of Example 23(a) (170mg, 0.29mmol) in dichloromethane (4ml) was then added. The mixture was then stirred at –40° C. for 10 min when a 0.5M aqueous solution of trisodium citrate (9ml) was added. After vigoursly stirring at room temperature the aqueous layer was separated, twice washed with dichloromethane then concentrated under reduced pressure. The residue was chromatographed on HP20SS eluting with water containing up to 2% acetonitrile. Pure fractions (as determined by HPLC)were combined and freeze dried to give the title compound as a white solid (71mg, 50%); ν_{max} (KBr) 1775, 1669 and 1607 (br) cm^{-1}; δ_H (D_2O) 1.54–1.70 (1H,m), 1.94–2.03 (2H,m), 2.15–2.28 (1H,m), 3.44 and 3.85 (2H, ABq, J 18Hz), 3.8–4.0 (2H,m), 3.99 (3H,s), 4.86 (1H,t, J 8Hz), 4.99 (1H,d, J 4.5Hz), 5.95 (1H,d, J 4.5Hz) and 7.01 (1H,s). [Mass spectrum: +ve ion (thioglycerol) MH^+(492)].

EXAMPLE 24

Sodium 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(tetrahydrofuran-2-yl)-1-carba-1-dethiaceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl 2-diazo-3-oxo-5-[(3SR,4RS)-3-phenylacetamidoazetidin-2-on-4-yl]pentanoate

A solution of 4-methoxybenzyl 3-oxo-5-[(3SR,4RS)-3-phenylacetamidoazetidin-2-on-4-yl]pentanoate (1.38g, 3.15mmol) [prepared by the method described for 4-nitrobenzyl 3-oxo-5-[(3SR,4RS)-3-phenoxyacetamidoazetidin-2-on-4-yl]pentanoate, C. Bodurow and M. A. Carr, *Tetrahedron Lett.*, 1989, 30, 4801] in acetonitrile (60ml) was treated with 4-toluenesulphonyl azide (870mg, 4.42mmol) and N,N-diisopropylethylamine (822μl, 4.73mmol) at 0° C. After 10 min., the ice-bath was removed and stirring was continued at room temperature for 2h. The reaction mixture was diluted with ethyl acetate and washed with brine. After drying over $MgSO_4$, the solvent was evaporated in vacuo and the residue purified by chromatography on silica gel eluting with ethyl acetate to yield the title compound (1.27g, 87%); ν_{max} (KBr) 2134, 1775, 1717, 1654, 1513 and 1304cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.59–1.70 (2H,m), 2.68–2.95 (2H,m), 3.55 and 3.65 (2H,

ABq, J 15.6Hz), 3.78 (1H,m), 3.82 (3H,s), 5.19 (2H,s), 5.25 (1H, ddd, J 8.1, 4.9, 1.0Hz), 6.25 (1H, br s, exch.), 6.49 (1H, br d, J 8.1Hz, exch.), 6.90 (2H,d, J 8.7Hz) and 7.23–7.70 (7H,m). [Mass spectrum : +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(487)].

(b) 4-Methoxybenzyl (6RS,7SR)-7-Phenylacetamido-3-(trifluoromethylsulphanyloxy)-1-carba-1-dethiaceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl 2-diazo-3-oxo-5-[(3SR, 4RS)-3-phenylacetamidoazetidin-2-on-4-yl]pentanoate (1.54g, 3.32mmol) in chloroform (40ml) was heated to reflux in the presence of a catalytic quantity of rhodium (II) acetate dimer. After heating for 45 min., the reaction mixture was cooled to 0° C. and treated sequentially with N,N-diisopropylethylamine (1.16ml, 6.66mmol) and trifluoromethanesulphonic anhydride (0.61ml, 3.65mmol). After stirring for 30 min at 0° C., the mixture was concentrated in vacuo. The residue was purified by chromatogrphy on silica gel eluting with 30, then 50% ethyl acetate in hexane yielding the title compound as an orange foam (1.20g, 64%); ν_{max} (CH_2Cl_2) 3417, 1783, 1733, 1684, 1516 and 1430cm^{-1}; δ_H (CDC_3, 250MHz) 1.45 (1H,m), 2.01 (1H,m), 2.56 (2H,m), 3.58 and 3.64 (2H, ABq, J 16.1Hz), 3.80 (3H,s), 3.87 (1H,m), 5.14–5.35 (3H,m), 5.89 (1H, br d, J 6.2Hz, exch), 6.87 (2H, d, J 8.7Hz) and 7.22–7.41 (7H,m), [Mass spectrum: +ve ion (ammonia) MH^+(569), MNH_4^+(586)].

(c) 4-Methoxybenzyl (6RS,7SR)-7-Phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6RS,7SR)-7-Phenylacetamido-3-(trifluoromethylsulphonyloxy)-1-carba-1-dethiaceph-3-em-4-carboxylate (1.13g, 199mmol) in THF (15ml) was treated with the cuprate species generated from (tetrahydrofuran-2-yl)-tri-n-butylstannane (1.97g, 5.46mmol), n-butyllithium (4.1ml of a 1.6M solution in hexane, 6.56mmol) and copper (I) bromide dimethylsulphide complex (565mg, 2.75mmol) as described in Example 11(a). Following work-up, the crude reaction product was purified by chromatography on silica gel eluting with 10, 20 and 30% ethyl acetate hexane. After elution of the 3-n-butylcarbacephem (340mg, 36%), the title compound was obtained as a mixture of diastereoisomers (478mg, 50%); (found: M+, 490.2096. C_{28} H_{30} N_2 O_6 requires M+490.2104.); ν_{max} (CH_2Cl_2) 3422, 1769, 1719, 1682, 1515 and 1389cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.45–2.70 (8H, m), 3.58 and 3.67 (2H, ABq, J 16.0Hz), 3.72–3.90 (3H,m), 3.80 (3H,s), 4.93 and 5.09 (together 1H, 2dd, J 8.9, 6.8 and 7.9, 7.9Hz), 5.13–5.28 (3H,m), 5.80 and 5.85 (together 1H, 2d, J 6.6, 7.7Hz, exch.), 6.89 (2H,d, J 8.7Hz) and 7.20–7.41 (7H,m)

(d) 4-Methoxybenzyl (6RS,7SR)-7-amino-3-(tetrahydrofuran-2-yl)-1-carba-1-dethiaceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6RS, 7SR)-7-phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (560mg, 1.14mmol) and N-methylmorpholine (250μl, 2.27mmol) in dichloromethane (15ml) was treated successively with phosphorus pentachloride (357mg, 1.71mmol) in dichloromethane (9ml), methanol (2.5ml) and water (5ml) as described in Example 1(f). Purification by chromatography on silica gel eluting with ethyl acetate and then 5% methanol in ethyl acetate yielded 4-methoxybenzyl (6RS,7SR)-7-amino-3-[(SR)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (166mg, 39%) as a colourless foam; ν_{max} (CH_2Cl_2) 3401, 1761, 1716, 1613 and 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.50–1.68 (2H,m), 1.85–1.97 (2H,m), 2.12–2.32 (2H,m), 2.35–2.45 (2H,m), 2.70 (2H, br s, exch.), 3.70–3.92 (3H,m), 3.78 (3H,s), 4.58 (1H,d, J 5.3Hz), 4.94 (1H,dd, J 8.8, 7.0Hz), 5.16 (2H,s), 6.87 (2H,d, J 8.7Hz) and 7.31 (2H,d, J 8.7Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(395)]. Further elution of the column yielded the more polar diastereoisomer 4-methoxybenzyl (6RS,7SR)-7-amino-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (132mg, 31%) as a pale yellow foam; ν_{max} (CH_2Cl_2) 3408, 1761, 1722, 1613 and 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.52–1.72 (2H,m), 1.80–2.00 (2H,m), 2.06–2.22 (2H,m), 2.50–2.78 (4H,m, 2H exch), 3.69–3.90 (3H,m), 3.78 (3H,s), 4.51 (1H,d, J 5.3Hz), 5.06 (1H,dd, J 7.8, 7.8Hz), 5.20 (2H,s), 6.87 (2H,d, J 8.6Hz) and 7.34 (2H,d, J 8.6Hz). [Mass spectrum : +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(395)].

(e) 4-Methoxybenzyl (6RS,7SR)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyaminoacetamido]-3-[(SR)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate

2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (99mg,0.49mmol) in DMF (5ml) was treated with methansulphonyl chloride (38μl,0.49mmol) and N,N-diisopropylethylamine (86μl, 0.49mmol) as described in Example 7(a). This was then treated successively with a solution of 4-methoxybenzyl (6RS,7SR)-7-amino-3-[(SR)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (160mg,0.43mmol) in DMF (5ml) and pyridine (40μl, 0.49mmol). After work-up, the product was purified by chromatography on silica gel eluting with ethyl acetate to yield the title compound (169mg, 71%); ν_{max} (KBr) 3313, 1763, 1717, 1676, 1612 and 1514cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.48–1.62 (2H,m), 1.83–1.98 (2H,m), 2.10–2.49 (6H,m, 2H exch.), 3.78–3.98 (3H,m), 3.79 (3H,s), 4.08 (3H,s), 4.98 (1H,dd, J 8.8, 6.9Hz), 5.13 and 5.20 (2H, ABq, J 12.2Hz), 5.48 (1H,dd, J 7.0, 5.0Hz), 6.89 (2H,d, J 8.6Hz), 7.00 (1H,s), 7.35 (2H,d, J 8.6Hz) and 7.82 (1H, br s, exch.). [Mass spectrum: +ve ion (3-nitrobenzylalcohol, sodium acetate) MH^+(556) MNa^+(578)].

(f) 4-methoxybenzyl (6RS,7SR)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate

2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (74mg, 0.37mmol) in DMF (5ml) was treated with methanesulphonyl chloride (29μl, 0.37mmol) and N,N-diisopropylethylamine (64μl, 0.37mmol) as described in Example 7(a). This was then treated successively with a solution of 4-methoxybenzyl (6RS,7SR)-7-amino-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (125mg, 0.34mmol) in DMF (5ml) and pyridine (30μl,0.37mmol). After work-up, the product was purified by triturating with diethyl ether to yield the title compound (148mg, 78%); ν_{max} (KBr) 3343, 1751, 1718, 1678 and 1515cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.25–1.30 (2H,m), 1.50–2.78 (8H,m, 2H exch.), 3.75–3.95 (3H,m), 3.78 (3H,s), 4.12 (3H,s), 5.12 (1H,dd, J 7.8, 7.4Hz), 5.19 (2H,s), 5.43 (1H,dd, J 7.3, 5.1Hz), 6.88 (2H, d, J 8.7Hz), 7.05 (1H,s), 7.35 (2H,d, J 8.7Hz) and 8.09 (1H, br s, exch.). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(556), MNa^+(578)].

(g) Sodium (6RS,7SR)-7-[2-(aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(SR)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6RS,7SR)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(SR)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (160mg, 0.29mmol) in dichloromethane (10ml) was added to a solution of aluminium chloride (115mg, 0.85mmol) in anisole (4.5ml) and dichloromethane (2.5ml) as described in Example 7(b). After quenching with trisodium citrate (0.5M,9ml) and subsequent work-up, the product was purified by chromotography on HP20SS eluting with water, then 1 and 2% THF in water. Fractions containing the product (h.p.l.c. analysis) were combined and freeze-dried to give the title compound (94mg, 71%); ν_{max} (KBr) 1745, 1663, 1595, 1532 and 1387cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1.38–1.55 (2H,m), 1.70–1.88 (3H,m), 1.97–2.16 (3H,m), 3.52–3.79 (3H,m), 3.82 (3H,s,), 4.95 (1H,dd, J 8.4, 7.0Hz), 5.22 (1H,dd, J 8.6, 4.9Hz), 6.73 (1H,s), 7.23 (2H, br s, exch.) and 9.18 (1H,d, J 8.6Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(458)].

(h) Sodium (6RS,7SR)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6RS,7SR)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyaminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]-1-carba-1-dethiaceph-3-em-4-carboxylate (140mg,0.25mmol) in dichloromethane (10ml) was added to a solution of aluminium chloride (101mg, 0.76mmol) in anisole (4.5ml) and dichloromethane (2.5ml) as described in Example 7(b). After quenching with trisodium citrate (0.5M, 8ml) and subsequent work-up, the product was purified by chromatography on HP20SS eluting with water, then 1 and 2% THF in water. Fractions containing the product (h.p.l.c. analysis) were combined and freeze-dried to give the title compound (54mg, 47%); ν_{max} (KBr) 1746, 1662, 1596, 1532 and 1387cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1,42–1.62 (2H,m), 1.68–1.88 (4H,m), 2.01 (1H, m), 2.27 (1H,m), 3.56–3.78 (3H,m), 3.85 (3H,s), 5.20 (2H, m), 6.75 (1H,s), 7.24 (2H,br s, exch.) and 9.25 (1H,d, J 8.7Hz). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(458)].

EXAMPLE 25

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1,1-dioxide

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]-ceph-3-em-4-carboxylate-1,1-dioxide

To an ice-cooled solution of 4-methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate (see Example 7) (300mg, 0.52mmol) in ethyl acetate (40ml) was added a solution of m-chloroperbenzoic acid (270mg, 1.56mmol) in ethyl acetate (10ml). The solution was stirred at room temperature for 1h and was then washed with dilute aqueous sodium hydrogen carbonate and water, dried (magnesium sulphate) and evaporated under reduced pressure. The residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give the title compound as a cream coloured solid (50mg, 15%); ν_{max} ($CHCl_3$) 1810, 1730 and 1690cm^{-1}; δ_H ($CDCl_3$) 1.52–1.70 (1H, m), 1.94–2.00 (2H, m), 2.41–2.48 (1H, m), 3.55 and 3.85 (2H, ABq, J 19Hz), 3.19 (3H, s), 3.3–3.43 (2H, m), 4.1 (3H, s), 4.90 (1H, d, J 5Hz), 4.97 (1H, t, J 7Hz), 5.20 (2H, s), 5.94–6.3 (2H, m, exch.), 6.20 (1H, dd, J 5, 10Hz), 6.91 (2H, d, J 8Hz), 7.06 (1H, s), 7.32 (2H, d, J 8Hz) and 7.86 (1H, d, J 10Hz, exch). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(606)].

(b) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamidol -3- [(S) -tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1,1-dioxide

The product from Example 25(a) was treated by the method of Example 23(b) to give the title compound (51%) as a freeze-dried white solid; ν_{max} (KBr) 1783, 1675 and 1610cm$_{-1}$; δ_H (d_6-DMSO) 1.45–1.50 (1H, m), 1.69–1.79 (2H, m), 2.00–2.11 (1H, m), 3.48 and 3.87 (2H, ABq, J 18Hz), 3.76 (3H, s), 3.50–3.86 (2H, m), 4.85 (1H, t, J 7Hz), 5.22 (H, d, J 5Hz), 5.61 (1H, dd, J 5, 7Hz), 6.79 (1H, s), 7.13 (2H, s, exch.) and 9.33 (1H, d, J 7Hz exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(508)].

EXAMPLE 26

(RS)-1-(Propan-2-yl)oxycarbonyloxyethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of (RS)-1-iodo-1-(propan-2-yl) oxycarbonyloxyethane (516mg) in 1-methyl-2-pyrrolidinone (2ml) was added dropwise over 45 mins to an ice-cold mixture of sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (237mg) and finely powdered potassium carbonate (276mg) in 1-methyl-2-pyrrolidinone (5ml). The mixture was stirred for an additional 15 mins, diluted with ethyl acetate, washed with water, brine, dried (magnesium sulphate), concentrated and flash chromatographed on silica gel eluting with 50, 60, 70, 80% ethyl acetate in hexane to give the title compound as a foam (58mg); ν_{max} ($CHCl_3$) 2960, 1787, 1760, 1682, 1633, 1519 and 1377cm^{-1}; δ($CDCl_3$, 250MHz) 1.20–2.50 (13H, m), 3.35–3.80 (2H, m), 3.80–4.20 (2H, m), 4.22 (3H, s), 4.83–5.10 (2H, m), 5.85–6.00 (1H, m), 6.85–7.08 (1H, m), 7.27 (1H, s) and 7.76 (1H, br, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa$^+$(606)].

EXAMPLE 27

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,2SR)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) Methyl 5-methyl-2-furoate

A solution of methyl 5-chloromethyl-2-furoate (5.0g, 28.7mmol) in ethyl acetate (40ml) was hydrogenated over 10% palladium on charcoal (50mg) for 3h. The catalyst was filtered off and washed with ethyl acetate. The combined filtrates were concentrated in vacuo and the residue purified by chromatography on silica gel eluting with 10% ethyl acetate in hexane to yield the title compound as a colourless oil (3.78g, 94%); ν_{max} (CH_2Cl_2) 1725, 1534, 1522, 1437 and 1311cm^{-1}; δ_H ($CDCl_3$, 90MHz) 2.38 (3H, s), 3.86 (3H, s),

6.12 (1H, br d, J 4Hz) and 7.07 (1H, d, J 4Hz). [Mass spectrum: M+(140)].

(b) 5-Methyl-2-furoic acid

Methyl 5-methyl-2-furoate (3.68g, 26.29mmol) in methanol (30ml) was treated with a solution of potassium hydroxide (2.80g, 50.0mmol) in water (15ml) and the mixture stirred for 2h at room temperature. The methanol was evaporated in vacuo, the residue dissolved in water and washed with ethyl acetate. The aqueous phase was acidified with 5N hydrochloric acid, and the product extracted with ethyl acetate (x3). The combined organic solutions were dried and concentrated to yield the title compound as a yellow solid (3.12g, 94%); m.p. 110–112° C.; (Found: M^+, 126.0312. $C_6H_6O_3$ requires M^+126.0317); ν_{max} (CH_2Cl_2) 3300–2700, 1688, 1524, 1424, 1305, 1210 and 1167cm^{-1}; δ_H ($CDCl_3$, 90MHz) 2.40 (3H, s), 6.15 (1H, d, J 4Hz) and 7.22 (1H, d, J 4Hz).

(c) 5-Methyl-2-tetrahydrofuroic acid

A solution of 5-methyl-2-furoic acid (3.65g, 28.97mmol) in ethyl acetate (60ml) was hydrogenated over 5% rhodium on carbon (250mg) until hydrogen uptake ceased. The catalyst was filtered off and washed with ethyl acetate. The combined filtrates were concentrated in vacuo to yield the title compound as a pale yellow oil (3.67g, 97%); ν_{max} (CH_2Cl_2) 3384, 3359, 1775, 1724 and 1355cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.35 (3H, d, J 6.1Hz), 1.53 (1H, m), 2.09 (1H, m), 2.17–2.40 (2H, m), 4.21 (1H, m) and 4.46 (1H, dd, J 8.9, 4.7Hz). [Mass spectrum: +ve ion (ammonia) MNH_4^+ (148)].

(d) 2-Bromoacetyl-5-methyltetrahydrofuran

A solution of 5-methyl-2-tetrahydrofuroic acid (1.80g, 13.85mmol) in dichloromethane (25ml) was treated with oxalyl chloride (2.4ml, 27.51mmol) in the presence of dimethylformamide (3 drops). After stirring for 1.25h, the solvent was evaporated in vacuo. The residue was re-dissolved in dichloromethane and concentrated again. Excess diazomethane was then bubbled through a solution of the resulting acid chloride in dichloromethane (30ml) at 0° C. When the addition was complete, the mixture was stirred for 10 min. at 0° C. and then treated with 48% aqueous hydrogen bromide (2.6ml, 15.41mmol). The mixture was stirred for 15 min. at room temperature, washed with water (x2), dried and concentrated in vacuo to yield the crude title compound as a brown oil (1.67g, 58%); ν_{max} (CH_2Cl_2) 1735, 1387 and 1086cm^{-1}; δ_H ($CDCl_3$, 90MHz) 1.33 (3H, d, J 6.0Hz), 1.48 (1H, m), 1.90–2.35 (3H, m), 4.10 (1H, m), 4.25 (2H, s) and 4.48 (1H, m).

(e) 4-Methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-(5-methyltetrahydrofuran-2-ylcarbonylmethylthio)-3-phenylacetamidoazetidin-2-on-1-yl]acetate

Toluene-4-sulphonic acid (3.42g, 17.98mmol) in water (8ml) was added to a solution of 4-methoxybenzyl (2RS)-2-hydroxy-2-[(1R,5R)-3-benzyl-4-thia-2,6-diazabicyclo [3.2.0]hept-2-en-7-on-6-yl]acetate (4.12g, 10.0mmol) in dichloromethane (20ml) and acetone (20ml). After stirring for 2.5h at room temperature, the reaction mixture was diluted with dichloromethane, washed with water (x2), dried and concentrated in vacuo to yield crude 4-methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-mercapto-3-phenylacetamidoazetidin-2-on-1-yl]acetate as a colourless foam. The crude thiol was dissolved in acetone (50ml) and treated with a solution of 2-bromoacetyl-5-methyltetrahydrofuran (1.67g, 8.1mmol) in acetone (5ml). After 10 min., potassium carbonate (687mg, 5.0mmol) was added, and the mixture stirred for a further 30 min. The reaction mixture was diluted with ethyl acetate, washed successively with water (x2) and brine, dried and concentrated. The residue was purified by chromatography on silica gel eluting with 50, 70 and 80% ethyl acetate in hexane to yield the title compound as a colourless foam (2.68g, 60%); ν_{max} (CH_2Cl_2) 3412, 1781, 1744, 1685 and 1515cm^{-1}. (Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(579)].

(f) 4-Methoxybenzyl 2-[(3R,4R)-4-(5-methyltetrahydrofuran-2-ylcarbonylmethylthio)-3-phenylacetamidoazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

A solution of thionyl chloride (530μl, 7.27mmol) in tetrahydrofuran (5ml) was added dropwise to the hydroxy compound (2.68g, 4.85mmol) and 2,6-lutidine (850μl, 7.29mmol) in tetrahydrofuran (30ml) at –20° C. After stirring for 30 min. the reaction mixture was filtered through a pad of celite and the filtrate concentrated in vacuo. Toluene was added and re-evaporated to yield 4-methoxybenzyl (RS)-2-chloro-2-[(3R,4R)-4-(5-methyltetrahydrofuran-2-ylcarbonylmethylthio)-3-phenyl-acetamidoazetidin-2-on-1-yl]acetate. The crude chloro-compound was dissolved in dioxan (40ml) and treated with tri-n-butylphosphine (2.7ml, 10.84mmol). After stirring for 30 min. at room temperature, the reaction mixture was diluted with ethyl acetate and washed successively with dilute sodium hydrogen carbonate solution, water and brine. The organic solution was dried, concentrated and then purified by chromatography on silica gel eluting with 50, 70 and 100% ethyl acetate in hexane to yield the title compound as a yellow foam (2.28g, 64%); ν_{max} (CH_2Cl_2) 3420, 1762, 1732, 1681 and 1515cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+741, MNa^+763].

(g) 4-Methoxybenzyl (6R,7R)-3-(5-methyltetrahydrofuran-2-yl)-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of the phosphorane (2.28g, 3.08mmol) and benzoic acid (10mg) in toluene (40ml) was heated in an oil bath at 130° C. for 16h under argon. The reaction mixture was cooled, concentrated and the residue purified by chromatography on silica gel eluting with 10, 20 and 40% ethyl acetate in hexane yielding a mixture of the title compound and some of the Δ2 isomer as a yellow oil (1.27g, 79%); (Found: M^+, 522.1813. $C_{28}H_{30}N_2O_6S_2$ requires M^+522.1825); ν_{max} (CH_2Cl_2) 3416, 1782, 1729, 1688, 1613 and 1515cm^{-1}.

(h) 4-Methoxybenzyl (6R,7R)-7-amino-3-(5-methyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

Phosphorus pentachloride (754mg, 3.62mmol) in dichloromethane (19ml) was added to 4-methoxybenzyl (6R,7R)-3-(5-methyltetrahydrofuran-2-yl)-7-phenylacetamidoceph-3-em-4-carboxylate (containing some of the Δ2-isomer) (1.26g, 2.41mmol) and N-methylmorpholine (531gl, 4.83mmol) in dichloromethane (15ml) at –25° C. The reaction was stirred at –10±5° C. for 45 min., then methanol (5ml) was added, and stirring was continued for 45 min. at room temperature. Water (10ml) was then added, and the

mixture vigorously stirred for a further 1h. After evaporation of the dichloromethane in vacuo, the pH of the aqueous residue was adjusted to 7 by the addition of ammonium hydroxide in the presence of ethyl acetate. The mixture was extracted with ethyl acetate (x2), dried and concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with 30, 50, 70, 80 and 100% ethyl acetate in hexane yielding 4-methoxybenzyl (6R,7R)-7-amino-3-[(5S, 2S)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (310mg, 32%) as a pale yellow foam; (Found: M^+, 404.1394. $C_{20}H_{24}N_2O_5S$ requires M^+404.1406); ν_{max} (CH_2Cl_2) 3412, 1776, 1721, 1613, 1516 and 1393cm^{-1}; δ_H ($CDCl_3$, 250MHz), 1.24 (3H, d, J 5.8Hz), 1.48 (1H, m), 1.69 (1H, m), 2.02 (3H, m, 2H exch.), 2.25 (1H, m), 3.45 and 3.60 (2H, ABq, J 17.7Hz), 3.78 (3H, s), 3.98 (1H, m), 4.88 (1H, d, J 5.0Hz), 4.93–5.04 (2H, m), 5.17 (2H, s), 6.87 (2H, d, J 8.6Hz), 7.32 (2H, d, J 8.6Hz).

Further elution of the column with ethyl acetate yielded the more polar diastereoisomer 4-methoxybenzyl (6R,7R)-7-amino-3-[(5R,2R)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (208mg, 21%) as a yellow foam; (Found: M^+404.1402. $C_{20}H_{24}N_2O_5S$ requires M^+404.1406); ν_{max} (CH_2Cl_2) 3411, 1776, 1727, 1613 and 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz), 1.24 (3H, d, J 6.1Hz), 1.48 (1H, m), 1.69 (, m), 1.92–2.08 (2H, m), 3.47 and 3.71 (2H, ABq, J 17.8Hz), 3.79 (3H, s), 4.00 (1H, dd, J 12.9, 6.4Hz), 4.83 (1H, d, J 4.8Hz), 4.92–5.17 (4H, m, 2H exch.), 5.19 (2H, s), 6.88 (2H, d, J 8.6Hz) and 7.32 (2H, d, J 8.6Hz).

Further elution of the column yielded the Δ2-cephems (142mg, 15%).

(i) 4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5S, 2S)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

2-(2-Aminothaizol-4-yl)-2-(Z)-methoxyiminoacetic acid (167mg, 0.83mmol) in DMF (5ml) was treated with methanesulphonyl chloride (64μl, 0.83mmol) and N,N-diisopropylethylamine (145μl, 0.83mmol) as described in Example 7(a). This was then treated successively with a solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(5S,2S)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (305mg, 0.75mmol) in DMF (5ml) and pyridine (67μl, 0.83mmol). After work-up the product was purified by chromatography on silica gel eluting with 50, 70 and 100% ethyl acetate in hexane to yield the title compound as a yellow foam (373mg, 85%); ν_{max} (CH_2Cl_2) 3389, 1784, 1724, 1689, 1606 and 1516cm^{-1}; δ_H ($CDCl_3$, 400MHz) 1.29 (3H, d, J 5.9Hz), 1.48 (1H, m), 1.69 (1H, m), 1.93 (2H, br s, exch.), 2.07 (1H, m), 2.29 (1H, m), 3.39 and 3.64 (2H, ABq, J 18.8Hz), 3.80 (3H, s), 4.00 (1H, dd, J 12.8, 6.4Hz), 4.10 (3H, s), 4.96 (1H, dd, J 7.7, 7.7Hz), 5.02 (1H, d, J 4.8Hz), 5.19 (2H, s), 5.84 (1H, br s, exch.), 5.94 (1H, dd, J 9.0, 4.8Hz), 6.89 (2H, d, J 8.5Hz) and 7.02 (1H, s), 7.35 (2H, d, J 8.5Hz). [Mass spectrum: +ve ion (ammonia) MH^+ (588)].

(j) 4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,2R)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (109mg, 0.54mmol) in DMF (3ml) was treated with methanesulphonyl chloride (42μl, 0.54mmol) and N,N-diisopropylethylamine (95μl, 0.55mmol) as described in Example 7(a). This was then treated successively with a solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(5R,2R)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (200mg, 0.50mmol) in DMF (10ml) and pyridine (44μl, 0.54mmol).

After work-up, the product was purified by triturating with diethyl ether to yield the title compound (214mg, 73%); ν_{max} (CH_2Cl_2) 3388, 1784, 1726, 1688, 1606 and 1516cm^{-1}; δ_H ($CDCl_3$, 400MHz) 1.26 (3H, d, J 6.0Hz), 1.46 (1H, m), 1.66 (1H, m), 1.87 (2H, br s, exch.), 2.00 (2H, m), 3.43 and 3.67 (2H, ABq, J 18.0Hz), 3.81 (3H, s), 4.00 (1H, dd, J 13.3, 6.3Hz), 4.09 (3H, s), 5.04 (1H, d, J 4.8Hz), 5.15–5.25 (3H, m), 5.55 (1H, br s, exch.), 5.89 (1H, dd, J 8.8, 4.8Hz), 6.90 (2H, d, J 8.6Hz), 6.98 (1H, s) and 7.34 (2H, d, J 8.6Hz). [Mass spectrum: +ve ion (ammonia) MH^+(588)].

(k) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5S,2S)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2- (Z)-methoxyiminoacetamido]-3-[(5S, 2S)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (370mg, 0.63mmol) in dichloromethane (10ml) was added to a solution of aluminium chloride (252mg, 1.89mmol) in anisole (10ml) and dichloromethane (5ml) as described in Example 7(b). After quenching with trisodium citrate (0.5M, 20ml) and subsequent work-up, the product was purified by chromatography on HP20SS eluting with water, then 1, 2, 3 and 4% THF in water. Fractions containing the product (h.p.l.c. analysis) were combined and freeze-dried to give the title compound (240mg, 78%); ν_{max} (KBr) 1762, 1670, 1602, 1532 and 1390cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1.15 (3H, d, J 6.0Hz), 1.41 (1H, m), 1.59 (1H, m), 1.85–2.08 (2H, m), 3.26 and 3.42 (2H, ABq, J 17.7Hz), 3.85 (3H, s), 3.87 (1H, m), 4.87 (1H, dd, J 7.3, 7.3Hz), 5.00 (1H, d, J 4.7Hz), 5.57 (1H, dd, J 8.0, 4.7Hz), 6.74 (1H, s), 7.22 (2H, s, exch.) and 9.51 (1H, d, J 8.0Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH^+(490)].

(l) Sodium (6R,7R)-7-[2-(2-aminothiazol)-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,2SR)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,2R)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (210mg, 0.36mmol) in 0.1M hydrochloric acid in 90% formic acid (3.6ml) was allowed to stand for 1h. Concentrated hydrochloric acid (2 drops) was then added, and the mixture left for a further 2.5h. After evaporating to dryness in vacuo, the residue was dissolved in water, the pH adjusted to 6.5 by addition of 1M sodium hydroxide solution and chromatographed on HP20SS eluting with 0, 1, 2, 3 and 4% THF in water. Fractions containing the product (h.p.l.c. analysis) were combined, concentrated and freeze-dried to give the title compound as a mixture of diastereoisomers (121mg, 69%); ν_{max} (KBr) 1763, 1663, 1598 and 1388cm–1; δ_H (d_6-DMSO, 250MHz) 1.10 and 1.16 (together 3H, 2d, J 6.0Hz), 1.27–2.17 (4H, m), 3.15–3.45 (together 2H, 2ABq), 3.84 (3H, s), 4.09 (1H, m), 4.93 and 4.95 (together 1H, 2d, J 4.6Hz), 5.02 and 5.18 (together 1H, 2dd, J 9.4, 5.9 and 7.6, 7.6Hz), 5.50 (1H, m), 6.74 and 6.76 (together 1H, 2s), 7.22 (2H, s, exch.) and 9.47 and 9.52 (together 1H, 2d, J 8.4Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH^+(490)].

EXAMPLE 28

Sodium (6R,7R)-7-[2-(furan-2-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl (6R,7R)-7-[2-(furan-2-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

2-(Furan-2-yl)-2-(Z)-methoxyiminoacetic acid (90mg) in dry DMF (4ml) was treated with N,N-diisopropylethylamine (0.1ml), cooled to −35° C., and treated with methanesulphonyl chloride (0.044ml) and the mixture stirred at −35° C. for 30 min.

A solution of the 4-methoxybenzyl (6R,7R)-7-amino-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (195mg) in dry DMF (3ml) was added followed by pyridine (0.044ml) and the mixture stirred at ice-bath temperature for a further 1h. The solution was diluted with excess ethyl acetate and the organic solution washed successively with 5% aqueous citric acid, saturated aqueous sodium bicarbonate solution and finally brine. After drying over anhydrous magnesium sulphate the solvent was evaporated. Chromatography of the residue on silica gel using ethyl acetate-hexane (1:1) as eluent gave the title compound as a pale yellow foam (190mg, 73%); ν_{max} 3400, 1785, 1725 and 1690cm^{-1}. [Mass spectrum: +ve ion (thioglycerol) MH$^+$ (542)].

(b) Sodium (6R,7R)-7-[2-furan-2-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Aluminium trichloride (130mg) was added to a solution of anisole (6ml) and dichloromethane (4ml) at −25° C. and the mixture stirred at −25° C. for 15min. The mixture was then cooled to −40° C., a solution of t product of Example 28(a) (180mg) in dichloromethane (4ml) added in one portion and stirred at −400° for 20min. The cooling bath was removed, trisodium citrate (10ml of an aqueous 0.5M solution) added and the mixture stirred vigorously for 20min. The aqueous layer was separated, washed twice with dichloromethane and concentrated under reduced pressure. The residue was chromatographed on HP20SS eluting with water-acetone mixtures. Fractions containing the product (t.l.c.; h.p.l.c. analysis) were combined, concentrated and freeze-dried to give the title compound as a white solid (95mg, 66%); μ_{max} (KBr) 1770, 1685 and 1600cm^{-1}; δ_H (D_2O) 1.65–1.85 (1H, m), 1.9–2.05 (2H, m), 2.08–2.15 (1H, m), 3.33 and 3.53 (2H, ABq, J 18Hz), 3.75–4.0 (2H, m), 3.96 (3H, s), 4.71 (1H, dd, J 8.3,6.9Hz), 5.2 (1H, d, J 4.5Hz), 5.73 (H, d, J 4.5Hz), 6.58 (1H, dd), 6.86 (1H, d) and 7.64 (1H, d).

EXAMPLE 29

Sodium (6R,7R)-7-[2-(2-aminothiazol)-2-(Z)-methoxyiminoacetamido]-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) (S)-2-Bromoacetyl-5,5-dimethyltetrahydrofuran

A solution of (S)-5,5-dimethyltetrahydrofuran-2-carboxylic acid (800mg, 5.56mmol) (I. Kitagawa, T. Nishino, M. Kobayashi, T. Matsuno, H. Akutsu and Y. Kyagaku, *Chem. Pharm. Bull.*, 1981, 29, 1942) in dichloromethane (25ml) was treated with oxalyl chloride (2.4ml, 27.51mmol) and dimethylformamide (3 drops). The mixture was stirred for 1h, evaporated in vacuo, dichloromethane added, and re-evaporated. The resulting acid chloride was dissolved in dichloromethane (25ml) and cooled in an ice-bath. Diazomethane was then passed into the solution as described in Example 14(a). When the addition was complete, 48% aqueous hydrogen bromide (2.6ml) was added, and the mixture stirred for a further 10 min. The solution was washed with water (x2), dried over $MgSO_4$ and concentrated in vacuo to yield the title compound as an orange oil (812mg, 66%); ν_{max} (CH_2Cl_2) 1767cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.28 (3H, s), 1.32 (3H, s), 1.78–2.68 (4H, m), 4.27 (2H, s) and 4.56 (1H, dd, J 8.2, 6.8Hz).

(b) 4-Methoxybenzyl (2RS)-2-Hydroxy-2-[(3R,4R)-4-[(S)-5,5-dimethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]acetate

4-Methoxybenzyl (RS)-2-hydroxy-2- [(1R, 5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl] acetate (3.3g, 8.0mmol) in 50% acetone/dichloromethane (32ml) was cleaved with 4-toluenesulphonic acid (2.74g, 14.4mmol) in water (6ml). This product was reacted with the crude bromide from Example 29(a) (808mg, 3.66mmol) in acetone (40ml) with potassium carbonate (550mg, 3.99mmol) as described in Example 6 (b). After work-up, the residue was purified by chromatography on silica gel eluting with 50, 70 and 90% ethyl acetate in hexane to yield the title compound (1.25g, 60%) as a yellow oil; ν_{max} (CH_2Cl_2) 3410, 1780, 1746, 1683, 1613 and 1515cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa$^+$(593)].

(c) 4-Methoxybenzyl 2-[(3R,4R)-4-[(S)-5,5-dimethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

The alcohol from Example 29(b) (1.25g, 2.19mmol) was treated with thionyl chloride (240μl, 3.29mmol) and 2,6-lutidine (383μl, 3.29mmol), followed by tri-n-butylphosphine (1.20ml, 4.82mmol) as described for Example 6(c). The product was purified by chromatography on silica gel eluting with 50, 70 and 100% ethyl acetate in hexane to yield the title compound (617mg, 37%) as a yellow foam; ν_{max} (CH_2Cl_2) 1763, 1680, 1608 and 1515cm^{-1}. [Mass spectrum: M$^+$(754)].

(d) 4-Methoxybenzyl (6R,7R)-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of the phosphorane from Example 29(c) (610mg, 0.81mmol) and benzoic acid (10mg) in toluene (20ml) was heated at reflux for 16h. After cooling, the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel eluting with 5 and 10% ethyl acetate in dichloromethane yielding the title compound as a yellow foam (240mg, 55%); (Found: M$^+$, 536.1978. $C_{29}H_{32}N_2O_6S$ requires M$^+$536.1981); ν_{max} (CH_2Cl_2) 3415, 1784, 1723, 1684 and 1515cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.22 (3H, s), 1.27 (3H, s), 1.62–1.81 (3H, m), 2.28 (1H, m), 3.30 and 3.56 (2H, ABq, J 18.8Hz), 3.60 and 3.69 (2H, ABq, J 16.3Hz), 3.82 (3H, s), 4.88 (1H, d, J 4.8Hz), 5.00 (1H, dd, J 8.6, 6.1Hz), 5.11 and 5.21 (2H, ABq, J 11.8Hz), 5.80 (1H, dd, J 9.1, 4.8Hz), 5.96 (1H, br d, J 9.1Hz, exch.), 6.88 (2H, d, J 8.7Hz) and 7.24–7.40 (7H, m).

(e) 4-Methoxybenzyl (6R,7R)-7-amino-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Phosphorus pentachloride (48mg, 0.23mmol) in dichloromethane (1.2ml) was added to 4-methoxybenzyl (6R,7R)-

3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate (93mg, 0.15mmol) and N-methylmorpholine (34μl, 0.31mmol) in dichloromethane (3ml) at −25° C. The reaction was stirred at −10±5° C. for 45min., then methanol (0.5ml) was added, and stirring continued for 45min. at room temperature. Water (1ml) was then added, and the mixture vigorously stirred for a further 1h. After evaporation of the dichloromethane in vacuo, the pH of the aqueous residue was adjusted to 7 by the addition of ammonium hydroxide in the presence of ethyl acetate. The mixture was extracted with ethyl acetate (x2), dried and concentrated in vacuo. The residue was purified by chromatography on silica gel eluting with 70% ethyl acetate in hexane yielding the title compound (25mg, 39%); (Found: M^+418.1566. $C_{21}H_{26}N_2O_5S$ requires M^+418.1562); ν_{max} (CH_2Cl_2) 2970, 1777, 1721, 1613 and 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.21 (3H, s), 1.27 (3H, s), 1.68–1.81 (3H, m), 2.25 (1H, m), 3.48 and 3.62 (2H, ABq, J 18.7Hz), 3.56 (2H, br s, exch.), 3.79 (3H, s), 4.73–5.25 (5H, m), 6.87 (2H, d, J 8.6Hz) and 7.30 (2H, d, J 8.6Hz).

(f) 4-Methoxybenzyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (13mg, 0.065mmol) in DMF (2ml) was treated with methanesulphonyl chloride (5ll, 0.064mmol) and N,N-diisopropylethylamine (11μl, 0.063mmol) as described in Example 7(a). This was then treated successively with a solution of the amine from Example 29(e) (25mg, 0.060mmol) in DMF (2ml) and pyridine (5μl, 0.062mmol). After work-up the product was purified by chromatography on silica gel eluting with 50, 70 and 100% ethyl acetate in hexane to yield the title compound (25mg, 70%) as a yellow foam; ν_{max} (CH_2Cl_2) 3389, 1784, 1722, 1690, 1607 and 1516cm^{-1}; δ_H ($CDCl_3$, 250MHz) 1.23 (3H, s), 1.29 (3H, s), 1.61–1.84 (3H, m), 2.31 (1H, m), 3.40 and 3.63 (2H, ABq, J 18.7Hz), 3.81 (3H, s), 4.20 (3H, s), 4.99 (1H, d, J 4.8Hz), 5.05 (1H, dd, J 8.1, 8.1Hz), 5.13 and 5.23 (2H, ABq, J 11.8Hz), 5.90 (1H, dd, J 8.9, 4.8Hz), 6.90 (2H, d, J 8.7Hz), 7.23 (1H, s), 7.34 (2H, d, J 8.7Hz), 7.50 (2H, br s, exch.) and 7.68 (1H, br d, J 8.9Hz, exch). [Mass spectrum: +ve ion (ammonia) MH^+(602)].

(g) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A solution of the ester from Example 29(f) (23mg, 0.038mmol) in dichloromethane (2ml) was added to a solution of aluminium chloride (15mg, 0.112mmol) in anisole (0.6ml) and dichloromethane (0.3ml) as described in Example 7(b). After quenching with trisodium citrate (0.5M, 1.3ml) and subsequent work-up, the product was purified by chromatography on HP20SS eluting with water, then 1, 2, 4 and 6% THF in water. Fractions containing the product (h.p.l.c. analysis) were combined and freeze-dried to give the title compound (13mg, 68%); ν_{max} (KBr) 1762, 1664, 1605 and 1529cm^{-1}; δ_H (d_6-DMSO, 250MHz) 1.13 (3H, s), 1.19 (3H, s), 1.59–1.73 (3H, m), 2.04 (1H, m), 3.22 and 3.37 (2H, ABq, J 17.5Hz), 3.83 (3H, s), 4.93 (1H, d, J 4.5Hz), 5.00 (1H, dd, J 7.9Hz), 5.52 (1H, dd, J 8.0, 4.5Hz), 6.75 (1H, s), 7.23 (2H, br s, exch.) and 9.48 (1H, d, J 8.0Hz, exch.). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(526)].

EXAMPLE 30

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5-methoxycarbonyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

(a) (2RS.5SR)-5-Methoxycarbonyltetrahydrofuran-2-yl carboxylic acid

A mixture of furan-2,5-dicarboxylic acid monomethyl ester (1.95g) and 5% rhodium on carbon (400mg) in ethyl acetate (50ml) was hydrogenated until hydrogen uptake ceased. The catalyst was filtered off and washed with ethyl acetate. The combined filtrates were evaporated to give (2.00g) of the title compound: ν_{max} (film) 3170, 1765 and 1720cm^{-1}; δ_H ($CDCl_3$) 1.95–2.65 (4H, m), 3.85 (3H, s) and 4.55–4.8 (2H, m).

(b) Methyl (2RS, 5SR)-5-(2-chloroacetyl) tetrahydro-2-furoate

Oxalyl chloride (1.55ml) was added to a stirred solution of (2RS, 5SR)-5-methoxycarbonyltetrahydrofuran-2-ylcarboxylic acid (2.00g) in dichloromethane (30ml). Dimethylformamide (1 drop) was added and the mixture stirred at room temperature for 1h, and then heated to reflux for 10 min. The mixture was cooled and the solvent removed on a rotary evaporator. Chloroform was then evaporated from the residue twice. The residue was dissolved in dichloromethane (100ml) and the solution cooled in an ice bath, then excess diazomethane was passed into the solution. The mixture was stirred at 0° C. for 15 min and then excess hydrogen chloride was passed into the solution. The solution was washed with brine, dried over magnesium sulphate and evaporated. The title compound (2.02g) was isolated by column chromatography of the residue using gradient elution (silica gel, 4:1 going to 1:1 hexane:ethyl acetate); ν_{max} ($CHCl_3$) 1740cm^{-1}; δ_H ($CDCl_3$) 1.9–2.5 (4H, m), 3.71 (3H, s), 4.45–4.8 (2H, m), 4.54 (1H, d, J 18Hz) and 4.89 (1H, d, J 18Hz).

(c) (3R, 4R)-4-8(2RS, 5SR)-5-Methoxycarbonyltetrahydrofuran-2-ylcarbonylmethylthio) -3-phenylacetamidoazetidin-2-one

Potassium carbonate (2.0g) was added to a stirred solution of (3R,4R)-4-mercapto-3-phenylacetamidoazetidin-2-one (2.31g) and methyl (2RS,5SR)-5-(2-chloroacetyl) tetrahydro-2-furoate (2.02g) in dimethylformamide (30ml). The mixture was stirred at room temperature for 1.5h and then partitioned between ethyl acetate and water. The aqueous phase was separated and extracted with ethyl acetate. The combined organic phases were washed three times with water, then brine, dried over magnesium sulphate and evaporated. The title compound (2.208g) was isolated by column chromatography of the residue (silica gel, ethyl acetate as eluent); ν_{max} ($CHCl_3$) 3410, 3335, 1777, 1736 and 1678cm^{-1}.

(d) 4-Methoxybenzyl (RS)-2-hydroxy-2-[4-[(2RS, 5SR)-5-methoxycarbonyltetrahydrofuran-2-ylcarbonylmethylthio)-3-phenylacetamidoazetidin-2-on-1-yl]acetate

4-Methoxybenzyl glyoxylate hydrate (1.50g) in dichloroethane (30ml) was heated at reflux for 1h using a Dean and Stark apparatus for heavy entrainers. The mixture was cooled to room temperature and then a solution of (3R,4R)-4-[(2RS,5SR)-5-methoxycarbonyltetrahydrofuran-2-

ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-one (2.208g) in dichloroethane (20ml) was added followed by triethylamine (0.1ml). The mixture was stirred at room temperature for 1h and then the solvents were evaporated. The title compound was obtained as a mixture of isomers (2.66g) by column chromatography of the residue using gradient elution (silica gel, 1:1 hexane:ethyl acetate going to neat ethyl acetate); ν_{max} ($CHCl_3$) 3412, 1776, 1741 and 1681cm^{-1}.

(e) 4-Methoxybenzyl 2-{(3R,4R)-4-[(2RS,5SR)-5-methoxycarbonyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl}-2-tri-n-butyl-phosphoranylideneacetate

A solution of thionyl chloride (0.51ml) in tetrahydrofuran (4ml) was added to a stirred solution of 4-methoxybenzyl (RS)-2-hydroxy-2-[(2R,4R)-4-[(2RS,5SR)-5-methoxycarbonyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]acetate (2.66g) and 2,6-lutidine (0.825ml) in tetrahydrofuran (21ml). The mixture was stirred at room temperature for 2h. The solid was filtered off and washed with tetrahydrofuran. The combined filtrates were evaporated and the residue was dissolved in toluene and the solvent evaporated. The residue was dissolved in dioxan (26ml) under argon and then tri-n-butylphosphine (2.6ml) was added. The mixture was stirred at room temperature for 0.5h and then ethyl acetate was added and the solution washed successively with sodium bicarbonate solution, water and brine. The solution was dried over magnesium sulphate and evaporated. The title compound (1.00g) was isolated by column chromatography of the residue using gradient elution (silica gel, 1:1, hexane:ethyl acetate, going to neat ethyl acetate); ν_{max} ($CHCl_3$) 3419, 1753, 1676 and 1612cm^{-1}.

(e) 4-Methoxybenzyl (6R,7R)-3-[(2RS,5SR)-5-methoxy-carbonyltetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl 2-{(3R,4R)-4-[(2RS,5SR)-5-methoxy-carbonyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl}-2-tri-n-butyl-phosphoranylideneacetate (1.00g) in toluene (100ml) was heated to reflux for 18h. The solvent was evaporated and the title compound (497mg) separated by column chromatography of the residue using gradient elution (silica gel, 1:1 hexane:ethyl acetate going to neat ethyl acetate); ν_{max} ($CHCl_3$) 3409, 1785, 1738 and 1684cm^{-1}.

(g) 4-Methoxybenzyl (6R.7R)-7-amino-3-(5-methoxy-carbonyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate

A solution of 4-methoxybenzyl (6R,7R)-3-[(2RS,5SR)-5-methoxycarbonyltetrahydrofuran-2-yl]-3-phenylacetamidoceph-3-em-4-carboxylate (497mg) in dichloromethane (7.2ml) was cooled to −15 to −16° C. and N-methylmorpholine (0.197ml) was added followed by phosphorus pentachloride in dichloromethane (7.0ml of a solution containing 40mg ml^{-1}). The mixture was stirred at the same temperature for 0.5h and then methanol (1.8ml) was added and the mixture stirred at room temperature for 0.5h. Water (2.4ml) was added and the mixture vigorously stirred for 0.5h. The dichloromethane was evaporated and the aqueous phase was stirred with ethyl acetate and the pH adjusted to 6.2 with dilute ammonia solution. The organic phase was washed with water, then brine, dried over magnesium sulphate and evaporated. The products were isolated by column chromatography using gradient elution (silica gel, 1:1 hexane:ethyl acetate going to neat ethyl acetate). Eluted first was 4-methoxybenzyl (6R, 7R)-7-amino-3-(2S, 5R)-5-methoxycarbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (41mg); ν_{max} ($CHCl_3$) 1778 and 1743cm^{-1}; δ_H ($CDCl_3$) 1.6–2.4 (4H, m), 2.68 (2H, br s) 3.65 (1H, d, J 18.7Hz), 3.74 (3H, s), 3.80 (3H, s), 3.89 (1H, d, J 18.7Hz), 4.49 (1H, dd, J 3.2, 8.9Hz), 4.79 (1H, d, J 4.7Hz), 4.93 (1H, d, J 4.7Hz), 5.06 (1H, dd, J 4.9, 9.8Hz), 5.17 (2H, s), 6.89 (2H, d, J 8.6Hz) and 7.33 (2H, d, J 8.5Hz). Eluted next was 4-methoxybenzyl (6R,7R)-7-amino-3-[(2R,5S)-5-methoxycarbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (126mg); ν_{max} ($CHCl_3$) 1777 and 1742cm^{-1}; δ_H ($CDCl_3$) 1.7–2.35 (4H, m), 2.44 (2H, br s), 3.59 (1H, d, J 17.8Hz), 3.73 (3H, s), 3.80 (3H, s), 3.98 (1H, d, J 17.8Hz), 4.51 (1H, dd, J 3.5, 8.8Hz), 4.72 (1H, d, J 4.9Hz), 4.94 (1H, d, J 4.9Hz), 5.15–5.30 (3H, m), 6.88 (2H, d, J 8.7Hz) and 7.34 (2H, d, J 8.7Hz).

(h) 4-Methoxybenzyl (6R,7R)-3-[(2R,5S)-5-methoxycarbonyltetrahydrofuran-2-yl]-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl) acetamido]ceph-3-em-4-carboxylate

A stirred solution of 2-(Z)methoxyimino-2-(2-tritylaminothiazol-4-yl)acetic acid hydrochloride (148mg) and N,N-diisopropylethylamine (0.107ml) in dimethylformamide (1ml) was cooled to −55 to −60° C. and methanesulphonyl chloride (0.024ml) was added. The mixture was stirred at the same temperature for 0.5h and then a solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(2R,5S)-5-methoxy-carbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (126mg) in dimethylformamide (1ml) was added followed by pyridine (0.023ml). The mixture was then stirred at 0° C. for 1h and then at room temperature for 0.5h. The mixture was partitioned between ethyl acetate and aqueous citric acid solution, and the organic phase was washed with water, then brine, dried over magnesium sulphate and evaporated. The title compound (100mg) was isolated by column chromatography of the residue (silica gel, 3:7 hexane:ethyl acetate as eluent); ν_{max} ($CHCl_3$) 3403, 1786, 1732 and 1681cm^{-1}; δ_H ($CDCl_3$) 1.66–2.36 (4H, m), 3.58 (1H, d, J 18.0Hz), 3.73 (3H, s), 3.81 (3H, s), 4.02 (1H, d, J 18.0Hz), 4.08 (3H, s), 4.53 (1H, dd, J 3.34, 8.91Hz), 5.02 (1H, d, J 4.8Hz), 5.18 (1H, d, J 11.8Hz), 5.24 (1H, d, J 12.0Hz), 5.30 (1H, dd, J 5.7, 9.9Hz), 5.86 (1H, dd, J 4.6, 8.6Hz), 6.72–6.83 (2H, m), 6.89 (2H, d, J 8.54Hz), 7.01 (1H, s) and 7.25–7.4 (17H, m).

(i) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2S,5S)-5-methoxycarbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Hydrochloric acid (0.12ml of 1N) was added to a stirred solution of 4-methoxybenzyl (6R,7R)-3-[(2R,5S)-5-methoxycarbonyltetrahydrofuran-2-yl]-7-[2-(Z)-methoxyimino-2-(2-tritylaminothiazol-4-yl)acetamido] ceph-3-em-4-carboxylate (100mg) in 98% formic acid (2ml). The mixture was stirred at room temperature for 0.5h and then concentrated hydrochloric acid (0.1ml) was added, and the mixture stirred for a further 1h at room temperature. The solid was then filtered off and the filter cake washed with 90% formic acid. The combined filtrates were evaporated and toluene evaporated from the residue twice. The residue was stirred with water and the pH adjusted to 6.2 with saturated aqueous sodium bicarbonate. The solution

was filtered and evaporated and the product isolated by column chromatography of the residue (HP20SS using water with increasing proportions of acetone as eluent). Fractions containing product were combined, evaporated and the residue dissolved in water (4ml) and freeze-dried to give a mixture (20.7mg) of the title compound; ν_{max} (KBr) 1762, 1669 and 1603cm^{-1}; δ_H[$(CD_3)_2SO$]1.54–2.35 (4H, m), 3.23 (1H, d, J 17.4Hz), 3.41 (1H, d, J 17.4Hz), 3.63 (3H, s), 3.83 (3H, s), 4.54 (1H, t, J 6.3Hz), 4.97 (1H, d, J 4.65Hz), 5.15 (1H, dd, J 5.9, 9.2Hz), 5.55 (1H, dd, J 4.6, 7.9Hz), 6.74 (1H, s), 7.23 (2H, s) and 9.48 (1H, d, J 8.1Hz), and the 3-(2R,5S) isomer; δ_H (inter alia), 3.66(s), 3.84(s), 4.42 (dd, J 3.5, 9.0Hz), 5.36 (dd, J 6.1, 9.7Hz), 6.76(s) and 9.53 (d, J 8.3Hz).

(j) 4-Methoxybenzyl (6R,7R)-3-[(2S,5R)-5-methoxycarbonyltetrahydrofuran-2-yl]-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]ceph-3-em-4-carboxylate

A stirred solution of 2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (20.1mg) and N,N-diisopropylethylamine (0.0176ml) in dimethylformamide (0.3ml) was cooled to −55 to −60° C. and methanesulphonyl chloride (0.0081ml) was added. The mixture was stirred at the same temperature for 0.5h and then a solution of 4-methoxybenzyl (6R,7R)-7-amino-3-[(2S,5R)-5-methoxycarbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (41mg) in dimethylformamide (0.3ml) was added followed by pyridine (0.0073ml). The mixture was then stored at 0° C. for 1h and then at room temperature for 0.5h. The reaction mixture was partitioned between ethyl acetate and aqueous citric acid solution and the organic phase washed with water and brine. The solution was dried over magnesium sulphate and evaporated, and the title compound (31mg) isolated by column chromatography of the residue (silica gel, ethyl acetate as eluent); ν_{max} ($CHCl_3$) 3496, 3397, 1784, 1733 and 1684cm^{-1}.

(k) Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2S,5R)-5-methoxycarbonyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A stirred solution of anisole (0.75ml) and dichloromethane (0.38ml) was cooled to −20° C. and aluminium chloride (19mg) was added. The mixture was stirred at the same temperature for 15min. and then cooled to −40° C., and then a solution of 4-methoxybenzyl (6R,7R)-3-[(2S,5R)-5-methoxycarbonyltetrahydrofuran-2-yl]-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]ceph-3-em-4-carboxylate (31mg) in dichloromethane (2.5ml) was added and the mixture stirred at the same temperature for 5min. Trisodium citrate (1.64ml of 0.5M solution) was then added and the mixture stirred for 10 min at room temperature. The aqueous phase was separated and washed twice with dichloromethane. The solution was evaporated and the product isolated by column chromatography of the residue (HP20SS, water with increasing proportions of acetone as eluent). Fractions containing product were combined, evaporated and the residue dissolved in water (3ml) and freeze dried to give the title compound (12mg); ν_{max} (KBr) 1762, 1670 and 1604cm^{-1}; δ_H [$(CD_3)_2SO$)]1.50–1.63 (1H, m), 1.90–2.26 (3H, m), 3.30–3.47 (2H, m), 3.65 (3H, s), 3.83 (3H, s), 4.39 (1H, dd, J 3.4, 8.7Hz), 4.98 (1H, d, J 4.8Hz), 5.0 (1H, dd, J 5.1, 9.8Hz), 5.52 (1H, dd, J 4.7, 8.2Hz), 6.75 (1H, s), 7.24 (2H, s) and 9.49 (1H, d, J 8.1Hz).

EXAMPLE 31

4-Methoxybenzyl (6R,7R)-3-(5-acetoxymethyltetrahydrofuran-2-yl)-7-phenylacetamidoceph-3-em-4-carboxylate

(a) 5-Acetoxymethylfuran-2-carboxylic acid

A mixture of 5-hydroxymethylfuran-2-carboxylic acid (5.90g), dry dichloromethane (100ml), pyridine (6.71ml), 4-dimethylaminopyridine (507mg), and acetic anhydride (4.21ml) was stirred for 2 hours at room temperature. The mixture was diluted with ethyl acetate and washed with 5M hydrochloric acid and brine (3 times), dried ($MgSO_4$), and evaporated. The residue was re-evaporated twice from dry toluene to give the title acid as a solid (5.00g); δ_H[$(CD_3)_2CO$]2.05 (3H, s), 5.11 (2H, s), 6.62 (1H, d, J 4Hz), 7.17 (1H, d, J 4Hz) and 8.31 (1H, br s).

(b) (2RS,5SR)-5-Acetoxymethyltetrahydrofuran-2-carboxylic acid

A solution of 5-acetoxymethylfuran-2-carboxylic acid (5.00g) in ethyl acetate (250ml) was stirred with decolourising charcoal (5.0g) for 10mins. The mixture was filtered through Kieselguhr and the residue was washed with ethyl acetate (30ml). The combined filtrates were hydrogenated over 5% rhodium on carbon (2.5g) until hydrogen uptake ceased. The mixture was filtered through Kieselguhr and the residue was washed with ethyl acetate (30ml). The combined filtrates were evaporated to give the title acid as an oil (3.64g); ν_{max} (Film) 3700–2800 and 1742cm^{-1}; δ_H[$(CD_3)_2CO$]1.4–2.5 and 2.00 (7H, m+s), 3.9–4.55 (4H, m) and 7.52 (1H, br s). [Mass spectrum: M^+(188), MH^+(189)].

(c) (2RS,55R)-2-Acetoxymethyl-5-bromoacetyltetrahydrofuran

Dry DMF (1 drop) was added to a stirred mixture of the acid from Example 31(b) (500mg) and oxalyl chloride (0.35ml) in dry dichloromethane (10ml). After stirring at room temperature for 1 hour the mixture was evaporated and the residue was re-evaporated from dry dichloromethane (2×2ml) to give the acid chloride as an oil; ν_{max} (Film) 1815, 1785 and 1744cm^{-1}.

The acid chloride was redissolved in dry dichloromethane (10ml) and treated sequentially with diazomethane (from N-methyl-N-nitrosotoluene-4-sulphonamide, 1.65g) and 48% aqueous hydrogen bromide (0.5ml) as for Example 6(a). After stirring at ice bath temperature for 10 mins the mixture was washed with water (2×3ml), dried ($MgSO_4$), and evaporated to approximately 5ml to provide a solution of the title bromoketone; ν_{max} (CH_2Cl_2) 1738cm^{-1}.

(d) (3R,4R)-4-[(2RS,5SR)-5-Acetoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-one

Anhydrous potassium carbonate (183mg) was added portionwise, over 1 minute, to a stirred, ice bath cooled mixture of (3R,4R)-4-mercapto-3-phenylacetamidoazetidin-2-one (627mg), dry DMF (5ml), and the dichloromethane solution of the bromoketone from Example 31(c). After 15 minutes the cooling bath was removed and the mixture was stirred for an additional 15mins. The mixture was diluted with ethyl acetate (30ml) and was washed with 5% citric acid (5ml), brine (5ml), saturated $NaHCO_3$ (5ml), and brine (3×5ml). The dried ($MgSO_4$) organic layer was evaporated and the residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures and neat ethyl acetate to give the title azetidinones as a gum (495mg); ν_{max} ($CHCl_3$) 3411, 3324 br, 1778, 1734 and 1673cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+ (443)].

(e) 4-Methoxybenzyl (RS)-2-[(3R,4R)-4-[(2RS,5SR)-5-acetoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]-2-hydroxyacetate

A mixture of the product from Example 31(d) (490mg), 4-methoxybenzyl glyoxylate monohydrate (272mg), ben-

zene (15ml), and dioxan (2ml) was heated for 1 hour at reflux with provision for the azeotropic removal of water (Dean and Stark apparatus containing molecular sieves 4A). The mixture was cooled to room temperature and treated with triethylamine (0.016ml). After stirring at room temperature for 1 hour the mixture was evaporated to give the title compound as a gum; ν_{max} ($CHCl_3$) 3613–3159, 1778, 1740 and 1676cm^{-1}.

(f) 4-Methoxybenzyl (RS)-2-[(3R,4R)-4-[(2RS, 5SR)-5-acetoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetitin-2-on-1-yl]-2-chloroacetate

The compound from Example 31(e) was dissolved in dry THF (20ml), cooled to −10° C., and treated with 2,6-lutidine (0.20ml) and thionyl chloride (0.13ml). After stirring at −10° C. for 10 minutes the mixture was diluted with dry toluene (10ml), filtered, and the residue was washed with dry toluene (10ml). The combined filtrates were evaporated and the residue was re-evaporated from dry toluene (2×3ml) to give the title compound as a gum; ν_{max} ($CHCl_3$) 1785, 1742 and 1681cm^{-1}.

(g) 4-Methoxybenzyl 2-[(3R,4R)-4-[(2RS,5SR)-5-acetoxymethyltetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

Tri-n-butylphosphine (0.64ml) was added, dropwise over 2 minutes, to a stirred solution of the compound from Example 31(f) in dry dioxan (10ml) at room temperature. After stirring at room temperature for 1 hour the mixture was evaporated and the residue was diluted with ethyl acetate and washed with saturated $NaHCO_3$ (5ml) and brine (3×5ml). The dried ($MgSO_4$) organic layer was evaporated and the residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures and neat ethyl acetate to give the title phosphorane as a gum (517mg); ν_{max} ($CHCl_3$) 3419, 1749, 1672 and 1611cm^{-1}.

(h) 4-Methoxybenzyl (6R,7R)-3-(5-acetoxymethyltetrahydrofuran-2-yl)-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of the phosphorane from Example 31(g) (517mg) in dry toluene (100ml) was heated at reflux under dry argon for 8 hours and evaporated. the residue was chromatographed on silica gel eluting with ethyl acetate/hexane mixtures to give two fractions. The less polar fraction contained 4-methoxybenzyl (6R,7R)-3-[(2S,5R)-5-acetoxymethyltetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate, a foam (105mg); ν_{max} ($CHCl_3$) 3410, 1784, 1726 and 1683cm^{-1}; δ_H ($CDCl_3$) 1.53–1.83 (3H, m), 2.09 (3H, s), 2.19–2.36 (1H, m), 3.30 and 3.55 (2H, ABq, J 18.9Hz), 3.60 and 3.69 (2H, ABq, J 16.2Hz), 3.81 (3H, s), 4.01–4.21 (3H, m), 4.90 (1H, d, J 4.8Hz), 4.96 (1H, dd, J 8.3, 6.7Hz), 5.12 and 5.17 (2H, AA'q, J 12.5Hz), 5.81 (1H, dd, J 9.2, 4.8Hz), 5.97 (1H, d, J 9.2Hz), 6.85–6.92 (2H, m), 7.25–7.41 (7H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(581), MNa^+(603)]. The more polar fraction contained 4-methoxybenzyl (6R,7R)-3-[(2R,5S)-5-acetoxymethyltetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate, a solid (191mg), m.p. 185–187° C. (needles ex ethyl acetate/hexane); ν_{max} ($CHCl_3$) 3407, 1785, 1731 and 1682cm^{-1}; δ_H ($CDCl_3$) 1.53–1.78 (2H, m), 1.90–2.05 (2H, m), 2.08 (3H, s), 3.35 and 3.57 (2H, ABq, J 18.0Hz), 3.61 and 3.69 (2H, ABq, J 16.2Hz), 3.80 (3H, s), 4.01–4.19 (3H, m), 4.91 (1H, d, J 4.8Hz), 5.12–5.27 (3H, m), 5.74 (1H, dd, J 4.8, 9.0Hz), 6.04 (1H, d, J 9.0Hz), 6.85–6.91 (2H, m), 7.25–7.41 (7H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(581), MNa^+(603)].

EXAMPLE 32

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

(a) (2RS,3SR)-3-Methyl-2-Tetrahydrofuroic acid

3-Methyl-2-furoic acid (5g) in ethyl acetate (100ml) and 5% rhodium on charcoal catalyst (0.5g) were hydrogenated at ambient temperature and the atmosphere for 6–7h. The catalyst was filtered off and replaced with a further quantity (lg) of catalyst. The reaction mixture was hydrogenated for a further 7h. This procedure was repeated again until no more hydrogen was absorbed. After filtration through kieselguhr and removal of solvent under reduced pressure, the title compound was obtained as a colourless oil (5.096g, quant.); ν_{max} (CH_2Cl_2) 3674, 3377(br), 1770 and 1722cm^{-1}; δ_H ($CHCl_3$) 1.08 (3H, d, J 7.1Hz), 1.72 (1H, m), 2.16 (1H, m), 2.68 (1H, m), 3.94 (1H, m), 4.18 (1H, m), 4.47 (1H, d, J 7.5Hz) and 9.42 (1H, v. br s, exch). [Mass spectrum: +ve ion (ammonia) MNH_4^+(148)].

(b) (2RS,3SR)-2-Bromoacetyl-3-Methyltetrahydrofuran

(2RS,3SR)-3-Methyl-2-tetrahydrofuroic acid 91.3g) was converted to the acid chloride with oxalyl chloride (2.54g, 1.75mls) in dichloromethane (20mls) as described in Example 1(a) . Diazomethane was passed through a solution of the acid chloride in dichloromethane (20mls), cooled in ice/water until i.r. analysis showed no starting material. Hydrobromic acid (2mls, 49% w/v aqueous solution), was added dropwise and the reaction mixture stirred vigorously for 10min. T.l.c. analysis showed complete conversion to the title compound. The solution was washed with water, brine and dried. The solvent was washed with water, brine and dried. The solvent was evaporated and the residue flash chromatographed on silica gel, eluting with 5 and then 10% ethyl acetate/hexane to give the product as an almost colourless oil, (1.621g, 79%); ν_{max} (CH_2Cl_2) 1732cm^{-1}; δ_H ($CDCl_3$) 0.96 (3H, d, J 7.2Hz), 1.70 (1H, m), 2.17 (1H, m), 2.70 (1H, m), 3.93 (1H, m), 4.12 and 4.25 (2H, ABq, J 14.7Hz) and 4.49 (1H, d, J 7.3Hz). [Mass spectrum: +ve ion (ammonia) MNH_4 (224)].

(c) 4-Methoxybenzyl (2RS)-2-Hydroxy-2-[(3R,4R)-3-phenylacetamido-4-[(2RS,3SR)-3-methyltetrahydrofuran-2-ylcarbonylmethylthio] azetidin-2-on-1-yl]acetate

4-Methoxybenzyl (2RS) -2-hydroxy-2-[(1R, 5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl] acetate (12.66g) was hydrolysed in 50% dichloromethane-acetone (80ml) with toluene-4-sulphonic acid hydrate (10.22g) in water (25ml) as described in Example 6(b). The crude thiol thus prepared (12.942g), in acetone (50ml) was treated with (2RS,3SR)-2-bromoacetyl-3-methyltetrahydrofuran (6.57g) in acetone (20ml) for 10min. at room temperature. Then potassium carbonate (2.08g) was added and stirring continued for 30min. The solution was diluted with ethyl acetate (200ml), washed with water (2x),

brine and then dried. Remvoal of solvent gave a yellow gum. Flash chromatography on silica gel eluting with 50, 60, 70, 80 and then 90% ethyl acetate-hexane afforded the title compound as a pale yellow foam (10.406g, 62%); ν_{max} (CH_2Cl_2) 3405(br), 1780, 1744, 1683 and 1613cm^{-1}.

(d) 4-Methoxybenzyl 2-[(3R,4R)-3-Phenylacetamido-4-[(2RS, 3SR)-3-methyltetrahydrofuran-2-ylcarbonylmethylthio] azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

4-Methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-3-phenylacetamido-4-[(2RS,3SR)-3-methyltetrahydrofuran-2-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate (10.406g) was converted to it's chloride with thionyl chloride (3.34g, 2.02ml) and 2,6-lutidine (3.00g, 3.25ml) in tetrahydrofuran (100ml) as described in Example 6(c). The crude chloride in dioxan (80ml) was then converted to the product with tri-n-butylphosphine (6.98ml) also described in 6(c). Flash chromatography on silica gel afforded the title compound as a foam (6.525g, 47%). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(763)].

(e) 4-Methoxybenzyl (6R,7R)-7-Phenylacetamido-3-[(2RS,3SR)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The phosphorane from Example 32(d), (6.525g) in xylene (120ml) heated under reflux for 6–7h. until t.l.c. analysis (ethyl acetate) showed no more starting material. Concentration and flash chromatography on silica gel eluting with 30 and then 40% ethyl acetate in hexane gave the diastereoisomer mixture of the product as a brown foam (1.293g, 28%); The ^{1}H n.m.r. spectrum showed substantial amounts of the Δ-2 isomeric cephems. The crude mixture in methanol (15ml) and dichloromethane (5ml) was treated at room temperature with a solution of sodium metaperiodate (0.636g) in water (5ml) overnight and then heated to about 60° C. for 1h. The precipitate was filtered off and the filtrate concentrated. The residue was partitioned between ethyl acetate-water. The organic phase was then dried and concentrated. The residual gum was purified by flash chromatography on silica gel, eluting with 50%, 70% ethyl acetate-hexane and then neat ethyl acetate. The sulphoxide derivative of the cephem was obtained as a yellow foam, (0.484g, 35%). This foam was dissolved in dimethylformamide (5ml), cooled, under argon, to −30° C. Phosphorus trichloride (0.239g, 0.152ml) was added and the solution stirred for ca. 1h. The solution was then diluted with ethyl acetate and washed with water (3x) and then brine. After drying and removal of solvent the crude title compound was obtained as a brown foam, (0.441g, 97%); a sample of the crude product was flash chromatographed on silica gel, eluting with 40%, 50% ethyl acetate-hexane and afforded the less polar isomer as a pale yellow foam; ν_{max} (CH_2Cl_2) 3415, 1783, 1722, 1688 and 1613cm^{-1}; δ_H ($CDCl_3$) 0.76 (3H, dr J 7.2Hz), 1.55 (1H, m), 2.14 (1H, m), 2.73 (1H, m), 3.26 and 3.55 (2H, ABq, J 18.6Hz), 3.60–3.77 (3H, m), 3.82 (3H, s), 4.04 (1H, m), 4.90 (1H, d, J 4.8Hz), 4.93 (1H, d, J 7.6Hz), 5.16 (2H, s), 5.80 (1H, dd, J 4.8, 9.0Hz), 6.04 (1H, d, J 9.0Hz), 6.88 (2H, m) and 7.24-7.41 (7H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate), MNa^+ (545)]. The second, more polar isomer was then eluted and isolated as a pale yellow solid; ν_{max} (CH_2Cl_2) 3414, 1782, 1726,.1688 and 1613cm^{-1}; δ_H ($CDCl_3$) 0.79 (1H, d, J 7.2Hz), 1.53 (1H, m), 2.07 (1H, m), 2.40 (1H, m), 3.24 and 3.49 (2H, ABq, J 18.1Hz), 3.57–3.75 (3H, m), 3.82 (3H, s), 3.91 (1H, m), 4.93 (1H, d, J 4.7Hz), 5.18 (2H, s), 5.26 (1H, d, J 6.7Hz), 5.71 (1H, dd, J 4.7, 9.0Hz), 6.08 (1H, d, J 9.0Hz), 6.88 (2H, d, J 8.7Hz) and 7.26–7.40 (7H, m). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate); MNa^+(545)].

(f) 4-Methoxybenzyl (6R,7R)-7-Amino-3-[(2RS, 3SR)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

4-Methoxybenzyl (6R, 7R) -7-phenylacetamido-3-[(2RS, 3SR)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate (0.692g) in dry dichloromethane (5ml) under argon was cooled to −20° C. This solution was then treated with 4-methylmorpholine (0.268g, 0.291ml) followed by a solution of phosphorus pentachloride in dichloromethane (0.415g in 10.37ml) in a rapid dropwise fashion. The solution was allowed to warm to −5° C. and maintained at this temperature for 0.5h. Methanol (5ml) was then added in one portion and the solution allowed to warm to room temperature, and stirred for 0.5h. Water (5ml) was then added and the solution rapidly stirred for a further 0.75h. The dichloromethane was evaporated at reduced pressure and replaced with ethyl acetate. The pH was adjusted to 7.5 with aqueous 880 ammonia. The aqueous phase was extracted with ethyl acetate and the combined organic layers washed with brine and dried. Removal of solvent and column chromatography on silica gel eluting with 60 and then 70% ethyl acetate in hexane afforded the (2S,3R) isomer of the title compound as a pale yellow foam, (0.197g, 37%); ν_{max} (CH_2Cl_2) 1777, 1720 and 1613cm^{-1}; δ_H ($CDCl_3$) 0.89 (3H, d, J 7.2Hz), 1.56 (1H, m), 2.14 (1H, m), 2.73 (1H, m), 3.37 and 3.57 (2H, ABq, J 18.0Hz), 3.73 (1H, m), 3.82 (3H, s), 4.05 (1H, m), 4.79 (1H, d, J 4.8Hz), 4.93 (1H, d, 4.8Hz), 4.98 (1H, d, J 7.6Hz), 5.17 (2H, s), 6.88 (2H, d, J 8.6Hz) and 7.33 (2H, d, J 8.6Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(405), MNa^+(427)].

The second compound to be eluted was the (2R,3S) isomer of the title compound, as a brown gum, (0.193g, 36%); ν_{max} (CH_2Cl_2) 1775, 1727 and 1613cm^{-1}; δ_H ($CDCl_3$) 0.84 (3H, d, J 7.0Hz), 1.54 (1H, m), 2.06 (1H, m), 2.45 (1H, m), 3.33 and 3.64 (2H, ABq, J 17.5Hz,), 3.71 (1H, m), 3.82 (3H, s), 3.92 (1H, m), 4.78 (1H, d, J 4.5Hz), 4.98 (1H, d, J 4.5Hz), 5.18 (2H, s), 5.29 (1H, d, J 8.1Hz), 6.88 (2H, d, J 8.6Hz) and 7.33 (2H, d, J 8.6 Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(405), MNa^+(427)]. Also isolated was a mixture of the isomers, (0.083g, 15%).

(g) 4-Methoxybenzyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2S,3R)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Methanesulphonyl chloride (0.04ml) was added to 2-(2-aminothiazol-4-yl)-2- (Z)-methoxyiminoacetic acid (0.103g) and N,N-diisopropylethylamine (0.089ml) in DMF (1ml) under argon at −50° C. The solution was maintained between −30° C. and −40° C. for 1h. A solution of the (2S,3R)-isomer from Example 34(f), (0.188g) and pyridine (0.038ml) in DMF (1ml) was added and the solution warmed to room temperature over 1h. The reaction mixture was diluted with ethyl acetate, washed successively with saturated sodium hydrogencarbonate, water, brine and then dried. After removal of solvent under vacuum, the residue was flash chromatographed on silica gel, eluting with 50, 70, 80 and then 90% ethyl acetate-hexane to give the title

compound as a waxy solid, (0.227g, 83%); ν_{max} (CH_2Cl_2) 3482, 3389, 1783, 1722, 1688, 1613 and 1516cm^{-1}; δ_H ($CDCl_3$) 0.89 (3H, d, J 7.1Hz), 1.57 (1H, m), 2.15 (1H, m), 2.76 (1H, m), 3.35 and 3.61 (2H, ABq, J 18.6Hz), 3.74 (1H, m), 3.82 (3H, s), 4.04 (1H, m), 4.10 (3H, s), 4.98 (1H, d, J 7.6Hz), 5.04 (1H, d, J 4.8Hz), 5.19 (2H, s), 5.22 (2H, br s, exch.), 5.94 (1H, dd, J 4.8, 8.9Hz, collapses to d, J 4.8Hz on exch.), 6.91 (2H, d, J 8.6Hz), 6.99 (1H, s), 7.20 (1H, d, J 8.9Hz, exch.) and 7.34 (1H, d, J 8.6Hz). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH$^+$(588), MNa$^+$(610)].

(h) 4-Methoxybenzyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2R,3S)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The procedure used in Example 32(g) was repeated for the (2R,3S) isomer from Examaple 32(h), (0.183g); with 2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetic acid (0.1g), N,N-diisopropylethylamine (0.087ml), methanesulphonyl chloride (0.039ml) and pyridine (0.037ml). After work up and purification the title compound was obtained as a pale yellow foam, (0.185g, 70%); ν_{max} (CH_2Cl_2) 3484, 3388, 1782, 1731, 1688, 1609 and 1516cm^{-1}; δ_H ($CDCl_3$) 0.84 (2H, d, J 7.1hz), 1.56 (1H, m), 2.09 (1H, m), 2.46 (1H, m), 3.33 and 3.60 (2H, ABq, J 17.8Hz), 3.73 (1H, m), 3.83 (3H, s), 3.93 (1H, m), 4.09 (3H, s), 5.07 (1H, d, J 4.6Hz), 5.21 (2H, s), 5.30 (1H, d, J 6.9Hz), 5.02 (2H, br s, exch.), 5.87 (1H, dd J 4.6, 8.7Hz collapses to d, J 4.6Hz on exch.), 6.90 (2H, d, J 8.7Hz), 6.98 (1H, s) and 7.35 (3H, d, J 8.7Hz overlapping m, exch). [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MN (588), MNa$^+$ (610)].

(i) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2S,3R)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

A mixture of dichloromethane (3ml) and anisole (6ml) under argon was cooled to −20° C. and aluminium trichloride (0.15g) was added. The solution was stirred for 0.25h and then cooled to −40° C. A solution of the cephem prepared in Example 32(g) in dichloromethane (6ml) was added in one portion. T.l.c. analysis (ethyl acetate) immediately after addition showed no starting material. A solution of trisodium citrate (12ml, 0.5M solution) was added and the mixture vigorously stirred for 10 minutes at room temperature. The aqueous phase was separated, washed twice with dichloromethane and concentrated to about 5ml. Column chromatography on HP20SS eluting with 0, 1, 2 and 4% tetrahydrofuran in water, followed by concentration and freeze-drying of the relevant fractions afforded the title compound as an amorphous white solid, (0.134g, 73%); ν_{max} (KBr) 1761, 1667, 1597 and 1531cm^{-1}; δ(d_6-DMSO) 0.86 (3H, d, J 7.1Hz), 1.48 (1H, m), 2.00 (1H, m), 2.56 (1H, m), 3.09 and 3.37 (2H, ABq, J 17.1Hz), 3.58 (1H, m), 3.84 (3H, s), 3.41 (1H, m), 4.92 (1H, d, J 7.7Hz), 4.96 (1H, d, J 4.6Hz), 5.53 (1H, dd, J 4.6, 8.1Hz, collapses to d, J 4.6Hz on exch.), 6.74 (1H, s), 7.24 (2H, br s, exch.) and 9.57 (1H, d, J 8.1Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(490), MNa$^+$(512)].

(j) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(2R,3S)-3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

The procedure used for Example 32(i) with dichloromethane (2.5ml), anisole (5ml), aluminium trichloride (0.12g) and the (2R,3S) isomer (0.178g) was employed. Following work up with trisodium citrate (10ml, 0.5M solution) the product was isolated and purified as described to give the title compound as an amorphous white solid, (0.117g, 79%); ν_{max} (KBr) 1762, 1665, 1597, 1532 and 1456cm^{-1}; δ_H (d_6-DMSO) 0.86 (3H, d, J 7.0Hz), 1.47 (1H, m), 2.00 (1H, m), 2.28 (1H, m), 3.18 and 3.38 (2H, ABq, J 16.9Hz), 3.58 (1H, m), 3.86 (3H, s), 3.93 (1H, m), 4.99 (1H, d, J 4.5Hz), 5.44 (1H, d, J 7.7Hz), 5.50 (1H, dd, J 4.5, 8.6Hz, collapses to d, J 4.5Hz on exch.), 6.76 (1H, s), 7.25 (2H, br s, exch.) and 9.50 (1H, d, J 8.6Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH$^+$(490), MNa$^+$(512)].

EXAMPLE 33

4-Methoxybenzyl (6R,7R)-3-[tetrahydropyran-4-yl]-7-phenylacetamidoceph-3-em-4-carboxylate

(a) 4-Methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-3-phenylacetamido-4-(tetrahydropyran-4-ylcarbonylmethylthio)azetidin-2-on-1-yl]acetate

Crude 4-methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-mercapto-3-phenylacetamidoazetidin-2-on-1-yl acetate {prepared from 4-methoxybenzyl (2RS)-2-hydroxy-2-[(1R, 5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl]acetate (8.35g, 20mmol)} was dissolved in acetone (25ml) and treated with a solution of 4-bromoacetyltetrahydropyran (G. H. Harnest and A. Burger, *J. Amer. Chem. Soc.*, 1943, 65, 370) (4.4g, 20mmol). After 20min., potassium carbonate (1.38g, 10mmol) was added and the mixture stirred again for a further 45min. Excess ethyl acetate was then added and the organic solution washed with water, brine and dried over anhydrous $MgSO_4$. Evaporation of solvent and chromatography of the residue on silica gel using 50% hexane in ethyl acetate to 100% ethyl acetate gave the title compound as a pale yellow foam (8.5g; 76%); ν_{max} ($CHCl_3$) 3420, 1780, 1750, 1680 and 1615cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa$^+$(579)].

(b) 4-Methoxybenzyl 2-[(3R,4R)-3-phenylacetamido-4-[tetrahydropyran-4-ylcarbonylmethylthio]azetidin-2-on-1-yl]-2-tri-n-butylphosphoranylidene acetate

A solution of thionylchloride (1.ml, 15mmole) in THF (10ml) was added dropwise to the hydroxy compound from Example 33(a) (5.56, 10mmol) and 2,6-lutidine (1.75ml, 15mmol) in THF (30ml) at −20° C. After stirring for 30min. the reaction was filtered through a pad of celite and the filtrate evaporated. Toluene was added and re-evaporated to yield 4-methoxybenzyl (RS)-2-chloro-2-[(3R,4R)-3-phenylacetamido-4-[tetrahydropyran-4-ylcarbonylmethylthio]azetidin-2-on-1-yl]acetate as a dark brown oil

The crude chloro compound was dissolved in dioxan (30ml) and treated with tri-n-butylphosphine (5.5ml, 22mmol). After stirring for 1h. at room temperature the reaction mixture was diluted with ethyl acetate and washed successively with dilute aqueous sodium bicarbonate solution, water and brine. After drying over anhydrous magnesium sulphate the solvent was evaporated. Chromatography of the residue on silica gel using ethyl acetate as eluent gave the title compound as a brown foam (6.2g, 84%); ν_{max} ($CHCl_3$) 3450, 1760, 1675, 1615 and 1510cm^{-1}. (Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH$^+$(741) MNa$^+$(763)].

(c) 4-Methoxybenzyl (6R,7R)-7-phenylacetamido-3-[tetrahydropyran-4-yl]ceph-3-em-4-carboxylate

A solution of the phosphorane from Example 33(b) (6g) and benzoic acid (20mg) in xylene (500ml) was heated at reflux for 44h. The reaction mixture was cooled, concentrated and the residue purified by chromatography on silica gel with 50% ethyl acetate in hexane to give the title compound as a mixture with the Δ^2 cephem (1:2) (1.24g); ν_{max} ($CHCl_3$) 3420, 1780, 1730, 1680 and 1615cm^{-1}; δ_H ($CDCl_3$), A^3 isomer, 1.20–1.90 (4H, m), 2.05–2.20 (1H, m), 3.10–3.40 (4H, m), 3.62 and 3.67 (2H, ABq, J 16.0Hz), 3.81 (3H, s), 3.85–4.05 (2H, m), 4.90 (1H, d, J 4.7Hz), 5.13 and 5.24 (2H, ABq, J 11.8Hz), 5.77 (1H, dd, J 4.7, 9.1Hz), 6.00 (1H, d, J 9.1Hz), 6.89 (2H, d, J 8.6Hz) and 7.25–7.45 (7H, m). [Mass spectrum: +ve ion (ammonia) 523 (MH^+), 540 (MNH_4^+)].

EXAMPLE 34

4-Methoxybenzyl (6R,7R)-3-[(2R,3R,4S)-3,4-dimethoxytetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate

(a) 1,4-Anhydro-2, 3-O,O-dimethyl-5,6-O-isopropylidene-D-qlucitol

1,4-Anhydro-5,6-O-isopropylidene-D-glucitol (S. Soltzberg, R. M. Goepp, Jr., and W. Freudenberg, *J. Amer. Chem. Soc.*, 1946, 68, 919) (8.74g, 43mmol), methyl iodide (11ml, 172mmol) and silver oxide (29.9g, 129mmol) in DMF (50ml) were stirred overnight, filtered through celite and evaporated in vacuo. The residue was extracted with ether, filtered through celite and evaporated to give the title compound as a colourless oil (8.26g, 83%); ν_{max} (CH_2Cl_2) 1675, 1457, 1381, 1270, 1216, 1108 and 1073cm^{-1}; δH ($CDCl_3$, 250MHz) 1.37 (3H, s), 1.43 (3H, s), 3.38 (3H, s), 3.45 (3H, s), 3.7–4.35 (8H, m).

(b) 1,4-Anhydro-2,3-O,O-dimethyl-D-glucitol

The product from Example 34(a) (8.26g) in ethanol (32ml) and water (8ml) was stirred with Amberlite IR 120 (H^+) (20g moist) for 4h. then filtered and evaporated to dryness to provide the title compound as an oil (6.50g, 95%); ν_{max} (CH_2Cl_2) 3583, 3460, 1462, 1108, 1179 and 1061cm^{-1}; δ_H ($CDCl_3$, 250MHz) 2.13 (br s, exch.), 3.39 (3H, s), 3.47 (3H, s), 3.65–4.0 (7H, m), 4.09 (1H, dd, J 4.63, 9.87Hz). [Mass spectrum: +ve ion (ammonia) MH^+(193), MNH_4^+ (210)].

(c) (2S,3R,4S)-3,4-Dimethoxytetrahydrofuran-2-yl-carboxaldehyde

Sodium metaperiodate (7.97g, 37mmol) in water (50ml) was added to an ice bath cooled solution of 1,4-anhydro-2, 3-O,O-dimethyl-D-glucitol (6.50g, 34mmol) in methanol (150ml) and then mixture stirred 0.5h then filtered and the filtrate evaporated in vacuo. The residue was extracted five times with dichloromethane then the combined extracts were dried ($MgSO_4$) and evaporated to give the crude aldehyde as a colourless oil (5.734g); ν_{max} (CH_2Cl_2) 3445, 1735, 1463, 1194 and 1120cm^{-1}; δ_H ($CDCl_3$, 250MHz), 3.38 (3H, s), 3.39 (3H, s), 3.94 (1H, d, J 3.87Hz), 4.02 (1H, d, J 9.99Hz), 4.14 (1H, d, J 4.77Hz), 4.20 (1H, dd, J 3.90, 10.07Hz), 4.39 (1H, dd, J 1.77, 4.72Hz) and 9.65 (1H, d, J 1.80Hz). [Mass spectrum: +ve ion (ammonia) MNH_4^+(178)].

(d) (2S,3R,4S)-3,4-Dimethoxytetrahydrofuran-2-ylcarboxylic acid

Jones reagent (R. G. Curtis, I. Heilbron, E. R. H. Jones and G. F. Woods, *J. Chem. Soc.*, 1953, 457) (11ml) was added dropwise to the aldehyde (5.73g) from Example 34(c) in acetone (125ml) cooled in an ice bath. After 10 minutes the orange solution was treated with propan-2-ol (2ml), stirred a further 10 minutes then diluted with ether (125ml), filtered through celite and evaporated in vacuo. The residue in dichloromethane was dried ($MgSO_4$), concentrated and flash chromatographed on silica gel eluting with 60, 70 and 80% ethyl acetate in hexane to give the title compound (4.68g, 72%) as a colourless oil; ν_{max} (CH_2Cl_2 3404(br), 1760, 1735, 1462, 1368, 1113, 1094 and 1056cm^{-1}; δ_H ($CDCl_3$, 250MHz) 3.40 (3H, s), 3.44 (3H, s), 3.94 (1H, d, J 3.83Hz), 4.00 (1H, d, J 9.90Hz), 4.07 (1H, d, J 4.16Hz) and 4.21 (1H, dd, J 3.83, 9.85Hz). [Mass spectrum: +ve ion (ammonia) MNH_4^+(194)].

(e) (2S,3R,4S)-2-Bromoacetyl-3,4-dimethoxytetrahydrofuran

A solution of (2S,3R,4S)-3,4-dimethoxytetrahydrofuran-2-carboxylic acid (3.0g, 17.0mmol) in dichloromethane (30ml) was treated with oxalyl chloride (3.0ml, 34.4mmol) and dimethylformamide (3 drops). The mixture was stirred for 1h., evaporated in vacuo, dichloromethane added, and re-evaporated. The resulting acid chloride was dissolved in dichloromethane (30ml) and cooled in an ice-bath. Diazomethane was then passed into the solution as described in Example 14(a). When the addition was complete, 48% aqueous hydrogen bromide (3.2ml) was added, and the mixture stirred for a further 10min. The solution was washed with water (x2), dried over $MgSO_4$ and concentrated in vacuo to yield the title compound (3.40g, 79%); (Found: M^+251.9986. $C_3H_13O_4Br$ requires 251.9997); ν_{max} (CH_2Cl_2) 1739cm^{-1}; δ_H ($CDCl_3$, 250MHz) 3.37 (3H, s), 3.39 (3H, s) and 3.41–4.70 (7H, series of m).

(f) 4-Methoxybenzyl (2RS)-2-hydroxy-2-[(3R,4R)-4-[(2S,3R,4S)-3,4-dimethoxytetrahydrofuran-2-ylcarbonylmethylthio]-3-phenylacetamidoazetidin-2-on-1-yl]acetate

4-Methoxybenzyl (RS)-2-hydroxy-2-[(1R,5R)-3-benzyl-4-thia-2,6-diazabicyclo[3.2.0]hept-2-en-7-on-6-yl]acetate (6.0g, 14.6mmol) in 50% acetone/dichloromethane (60ml) was cleaved with 4-toluenesulphonic acid (5.0g, 26.3mmol) in water (12ml). The product was reacted with crude bromide from Example 34(e) (3.40g, 13.4mmol) in acetone (70ml) followed by potassium carbonate (1.0g, 7.2mmol) as described in Example 6(b). After work-up, the residue was purified by chromatography on silica gel eluting with 50, 70 and 100% ethyl acetate in hexane to yield the title compound (3.40g, 42%); ν_{max} (CH_2Cl_2) 3400, 1781, 1735, 1682, 1613 and 1516cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MNa^+(625)].

(g) 4-Methoxybenzyl 2-[(3R,4R)-4-[(2S,3R,4S)-3,4-dimethoxytetrahydrofuran-2-ylcarbonylmethylthiol-3-phenylacetamidoazetidin-2-on-1-yl]-2-tri-n-butylphosphoranylideneacetate

The alcohol from Example 34(f) (3.35g, 5.56mmol) was treated with thionyl chloride (623μl, 8.54mmol) and 2,6-lutidine (995μl, 8.54mmol), followed by tri-n-butylphosphine (3.12ml, 12.52mmol) as described for Example 6(c). The product was purified by chromatography on silica gel eluting with 0 and 10% methanol in ethyl acetate to yield the title compound (2.68g, 61%); ν_{max} (CH_2Cl_2) 1761, 1682, 1613 and 1515cm^{-1}. [Mass spectrum: +ve ion (3-nitrobenzyl alcohol, sodium acetate) MH^+(787), MNa^+(809)].

(h) 4-Methoxybenzyl (6R,7R)-3-[(2R,3R,4S)-3,4-dimethoxytetrahydrofuran-2-yl]-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of the phosphorane from Example 34(g) (2.60g, 3.30mmol) and benzoic acid (10mg) in toluene (40ml) was heated to reflux for 16h. After cooling, the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel eluting with 10, 30 and 50% ethyl acetate in hexane to yield the title compound contaminated with the Δ2-isomer (296mg, 16%); ν_{max} (CH_2Cl_2) 3418, 1783, 1732, 1682, 1612 and 1515cm^{-1}; δ_H, Δ3-isomer ($CDCl_3$, 250MHz) 3.25 (3H, s), 3.31 (3H, s), 3.32–4.16 (8H, series of m), 3.80 (3H, s), 4.92 (1H, d, J 4.8Hz), 4.98–5.28 (3H, m), 5.77 (1H, dd, J 9.2, 4.8Hz), 6.00 (1H, br d, J 9.2Hz, exch.), 6.88 (2H, d, J 8.6Hz) and 7.22–7.41 (7H, m). [Mass spectrum: M^+(568)].

EXAMPLE 35

2-Ethoxycarbonyl-Z-but-2-enyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate

Sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl] ceph-3-em-4-carboxylate, (0.35g) in 1-methyl-2-pyrrolidinone (4ml) was treated with a solution of ethyl (Z)-2-bromomethylbut-2-enoate, (0.16g) in 1-methyl-2-pyrrolidinone (1ml) and stirred at ambient temperature overnight. The solution was diluted with ethyl acetate and washed with water (3x), brine and then dried. After removal of solvent in vacuo, the residue was purified by flash chromatography on silica gel, eluting with 70, 90% ethyl acetate-hexane and then ethyl acetate. The title compound was obtained as a pale yellow foam (0.368g, 86%); ν_{max} (CH_2Cl_2) 3480, 3389, 3320, 1781, 1726, 1682, 1606 and 1532cm^{-1}; δ_H ($CDCl_3$) 1.30 (4H, t, J 7.1Hz, overlapping M), 1.66 (1H, m), 1.97 (3H, d, J 7.3Hz), 2.35 (1H, m), 3.33 and 3.64 (2H, ABq, J 18.7Hz), 3.88 (2H, m), 4.08 (3H, s), 4.22 (2H, q, J 7.1Hz), 4.93 (1H, m), 5.05 (3H, m), 5.80 (2H, br s, exch.), 5.99 (1H, dd, J 4.8, 9.0Hz, collapses to d, J 4.8Hz on exch.), 6.83 (1H, s), 7.21 (1H, q, J 7.3Hz) and 7.73 (1H, d, J 9.0Hz, exch.). [Mass spectrum: +ve ion (thioglycerol) MH^+(580)].

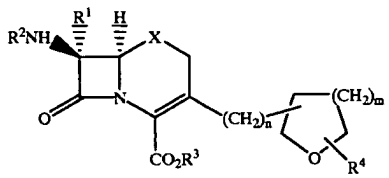
In Vitro Biological Data MIC (μg/ml)

	Organism	
Example No.	*E.coli* (NCTC 1048)	*S.aureus* (Oxford)
1	0.50	1.00
3	2.00	1.00
5	0.50	0.25
7	0.50	1.00
9	1.00	0.50
13	1.00	4.00
17	1.00	2.00
18	16.00	1.00
19	4.00	2.00
21	0.25	8.00
22	8.00	0.25
24	0.12	1.00
27	4.00	1.00
28	>32	0.50

We claim:

1. A compound of formula (I) or a salt thereof:

(I)



wherein R^1 is hydrogen, methoxy or formamido;

R is an acyl group;

CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group;

R^4 represents hydrogen or up to four substituents selected from alkyl, alkenyl, alkynyl, alkoxy, hydroxy, halogen, amino, alkylamino, acylamino, dialkylamino, CO_2R, $CONR_2$, SO_2NR_2 (where R is hydrogen or C_{1-6} alkyl) and aryl, which may be the same or different and wherein any R^4 alkyl substituent is optionally substituted by any other R^4 substituent;

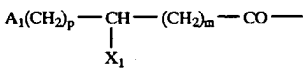
X is S, SO, SO_2, O or CH_2;

m is 1 or 2;

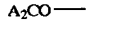
n is 0;

"acyl" is selected from the group consisting of formula (a) to (f):

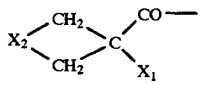
(a)



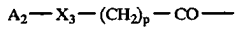
(b)



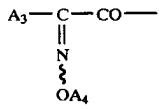
(c)



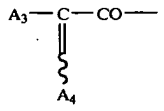
(d)



(e)



(f)



wherein p is 0, 1 or 2;

m is 0, 1 or 2;

A_1 is (C_{1-6})alkyl, substituted (C_{1-6})alkyl, (C_{3-6}) cycloalkyl, cyclohexenyl, cyclohexadienyl, or an aromatic group;

X_1 is a hydrogen or halogen atom, a carboxylic acid, carboxylic ester, sulphonic acid, azido, tetrazolyl, hydroxy, acyloxy, amino, ureido, acylamino, heterocyclylamino, guanidino or acylureido group;

A_2 is an aromatic or a substituted alkyl group, or a substituted dithietane;

X_2 is a $—CH_2OCH_2—$, $—CH_2SCH_2—$ or alkylene group;

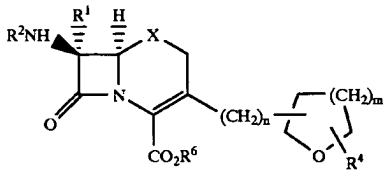
X_3 is an oxygen or sulphur atom;

A_3 is an aryl or heteroaryl group; and

A_4 is hydrogen, (C_{1-6})alkyl, (C_{3-8})cycloalkyl, (C_{3-8})cycloalkyl(C_{1-6})alkyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, (C_{2-6})alkenyl, carboxy(C_{1-6})alkyl, (C_{2-6})alkynyl, aryl or (C_{1-6})alkyl substituted by up to three aryl groups.

2. A compound as claimed in claim 1 having the formula (Ia):

(Ia)



wherein R^1, R^2, R^4, m, n and X are as defined with respect to formula (I) in claim 1 and the group CO_2R^6 is CO_2R^3 where CO_2R^3 is a carboxy group or a carboxylate anion, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof.

3. A compound as claimed in claim 1 wherein R^1 is hydrogen.

4. A compound as claimed in claim 1 wherein A_1 is optionally substituted phenyl, X_1 is hydrogen or amino, A_2 is optionally substituted phenyl, X_3 is oxygen, A_3 is aminothiazolyl, aminothiadiazolyl or furyl, and R^4 is hydrogen, C_{1-6} alkyl, or carboxy C_{1-6} alkyl.

5. A compound as claimed in claim 1 wherein CO_2R^3 is carboxy or a carboxylate anion or R^3 is t-butyl, 4-methoxybenzyl, diphenylmethyl, acetoxymethyl, acetoxyethyl, pivaloyloxymethyl, propan-2-yloxycarbonyloxyethyl or 2-ethoxycarbonyl-but-2-enyl.

6. A compound as claimed in claim 1 wherein the cyclic ether group bonded to the 3-position of the cephalosporin nucleus is unsubstituted or substituted by up to three substituents selected from C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkoxycarbonyl, C_{1-6} alkanoyloxy C_{1-6} alkyl or C_{1-6} alkoxy C_{1-6} alkyl.

7. A compound as claimed in claim 1 wherein m is 1.

8. A compound as claimed in claim 1 wherein the cyclic ether group is a tetrahydrofuran-2-yl or a tetrahydropyran-2-yl group.

9. A compound as claimed in claim 1 selected from the group consisting of:

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

pivaloyloxymethyl(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate;

pivaloyloxymethyl(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydropyran-2-yl]ceph-3-em-4-carboxylate;

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylic acid;

sodium (6R, 7R) -7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

pivaloyloxymethyl(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

pivaloyloxymethyl(6R,7R) -7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(R)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

diphenylmethyl (6R,7R) -7-phenylacetamido-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R, 7R) -7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-3-yl]ceph-3-em-4-carboxylate;

acetoxymethyl(6R, 7R) -7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5-methoxymethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-(Z)-pent-2-enamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

(RS)-1-acetoxyethyl(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-carboxymethoxyiminoacetamido]-3-[(RS)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylic acid disodium salt;

sodium (6R, 7R) -7-[(R)-2-amino-2-(4-hydrophenyl)-acetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (1S,6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1-oxide;

sodium 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(tetrahydrofuran-2-yl)-1-carba-1-dethiaceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate-1,1-dioxide;

(RS)-1-(propan-2-yl)oxycarbonyloxyethyl (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(5R,2SR)-5-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(furan-2-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(S)-5,5-dimethyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(5)-methoxycarbonyltetrahydrofuran-2-yl)ceph-3-em-4-carboxylate;

sodium (6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[3-methyltetrahydrofuran-2-yl]ceph-3-em-4-carboxylate; and

2-ethoxycarbonyl-(Z)-but-2-enyl (6R,7R)-7-[2-(2-aminothiazol-4-yl) -2-(Z)-methoxyiminoacetamido]-3-[(S)-tetrahydrofuran-2-yl]ceph-3-em-4-carboxylate.

10. A pharmaceutical composition comprising a compound of claim **2** or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition as claimed in claim **10** further comprising a β-lactamase inhibitor.

12. A method of treating bacterial infections in humans and animals which comprises administering a therapeutically effective amount of a compound of claim **2** or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof to a human or animal.

* * * * *

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,020,329 Page 1 of 1
DATED : February 1, 2000
INVENTOR(S) : John Hargreaves Bateson, George Burton and Stephen Christopher Martin Fell

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Lines 5-8, the paragraph should read
-- This application is a continuation of U.S. Application No. 08/470,786, filed June 6, 1995, now abandoned, which was a continuation of U.S. Application No. 07/934,667, filed January 22, 1993, which was the National Stage of International Application No. PCT/GB91/01228, filed July 22, 1991. --

Column 70,
Line 15, "R is an acyl group" should read -- R^2 is an acyl group --

Signed and Sealed this

Eleventh Day of June, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

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PFIZER INC
PATENT DEPARTMENT
235 EAST 42ND STREET
NEW YORK NY 10017-5755

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,020,329	**$890.00**	**$0.00**	**06/27/03**	**08/958,864**	**02/01/00**	**10/20/97**	**04**	**NO**	**PC-9137B**

EXHIBIT D

UNITED STATES PATENT AND TRADEMARK OFFICE

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Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,020,329	**$2,300.00**	**$0.00**	**06/21/07**	**08/958,864**	**02/01/00**	**10/20/97**	**08**	**NO**	**PC-9137B**

EXHIBIT E

Convenia (cefovecin sodium)

NADA 141-285

Chronology of Review of CONVENIA (Cefovecin sodium) File for Dogs (INAD 10-612):

File Section	Source	Date	Description	Time Under Review (Days)
INAD				
INAD dog	Pfizer	8/5/1999	Original request to open INAD for Dog.	
INAD dog	CVM A0000	11/16/1999	CVM assigned file numbers for INAD	103
Safety				
Safety (tolerance)	Pfizer	11/10/1999	Pfizer requests review of drug tolerance protocol 99-1990-01 entitled "Acute Safety Toleration".	
Safety (tolerance)	CVM E0002	1/21/2000	CVM finds the Acute Safety Toleration Protocol acceptable as submitted making revisions (listed).	72
Safety (tolerance)	Pfizer	5/10/2000	Submission of the Revised Final Drug Tolerance Protocol 1461N-60-00-419.	
Safety (tolerance)	CVM E0006	7/24/2000	CVM finds the Final Revised target Animal Safety Drug Tolerance protocol acceptable	75

Safety (MOS)	Pfizer	5/22/2000	Pfizer requests review of "Exploratory Margin of Safety and Injection Site Toleration"	
Safety (MOS)	CVM E0007	7/31/2000	CVM finds the Exploratory MOS and Injection Site Toleration Protocol acceptable as submitted	70
Safety (Stat analysis)	Pfizer	12/11/2000	Pfizer requests review and comments on the statistical approach for evaluating clinical pathology variables assoc. w/ TAS studies.	
Safety (Stat analysis)	CVM A0000	8/30/2001	CVM has reviewed the Statistical Approach for evaluating clinical pathology variables associated w/ TAS studies (as submitted 12/11/2000) and has requested information be provided in support.	262
Safety (Technical Section)	Pfizer	8/29/2002	Submission of Target Animal Safety Technical Section for Dogs	
Safety (Technical Section)	CVM/ P0156	3/24/2003	CVM considers the Target Animal Safety Technical Section to be complete for the purpose of recommending approval of a New Animal Drug Application	207

Safety (Technical Section)	Pfizer	6/30/2003	Pfizer submission of a response to CVM's comments dated 24 March 2003 on the Target Animal Safety Technical Section	
Safety (Technical Section)	CVM G0190	10/10/2003	CVM considers possible labeling issues raised under the Target Animal Safety review to be satisfactorily addressed.	102
Safety (Technical Section)	Pfizer	10/29/2004	Submission of further response to CVM's March 24, 2003 review of FOI for the TAS TS updating the FOI and correcting a statistical error	
Safety (Technical Section)	CVM G-0199	1/27/2005	CVM provides a copy of the FOI Summary reflecting the requested changes in the safety section in statistical significance for injection site swellings identified in the study.	90
Efficacy				
Efficacy Clinical Field Trial Protocol	Pfizer	12/22/2000	Pfizer requests review of "Efficacy & Safety of UK-287,074 in Treatment of Skin and Soft Tissue Infections in Dogs" study 1163C-60-00-468.	
Efficacy Clinical Field Trial Protocol	CVM E0012	4/5/2001	CVM finds the clinical field efficacy protocol for SST not acceptable and requires revisions.	104

Efficacy Clinical Field Trial Protocol	Pfizer	6/14/2001	50 Day Review Request for SST Pivotal Clinical Trial Protocol revised in accordance w/ CVM's comments by letter and meeting.	
Efficacy Clinical Field Trial Protocol	CVM/ E0018	8/16/2001	CVM finds the SST Efficacy and Safety Protocol acceptable	63
Efficacy Technical Section	Pfizer	9/5/2003	Submission of Effectiveness Technical Section for Skin Infections in Dogs.	
Efficacy Technical Section	CVM/ P0191, T0192	12/31/2003	CVM incompletes the Effectiveness Technical Section (SSTI Claim)	117
Efficacy Technical Section	Pfizer	4/21/2004	Submission of an interim update of our findings regarding the microbiological data performed by LabCorp and a proposal to resolve issues with this data.	
Efficacy Technical Section	CVM G-0196	8/4/2004	CVM finds the proposed plan of action to be reasonable.	105
Efficacy Technical Section	Pfizer	12/31/2004	Submission of an amendment to the Effectiveness Technical Section	
Efficacy Technical Section	CVM P0200	10/3/2005	CVM incompletes the Effectiveness Technical Section (EFF TS) for SSTI.	276

Efficacy Technical Section	Pfizer	12/24/2005	Submission of a response to CVM's Incomplete Letter dated 3 October 2005	
Efficacy Technical Section	CVM P-0213	7/14/2006	CVM Incompletes the Skin Infection (SI) Effectiveness Technical Section submitted on 24 December 2005 for the treatment of skin infections	202
Efficacy Technical Section	Pfizer	5/11/2007	Submission of a response to the gap in safety data presented by CVM in correspondence dated 14 July 2006 and subsequent conference on 14 September	
Efficacy Technical Section	CVM P-0232-EF	11/21/2007	CVM incompletes 11 May 2007 submission because an effectiveness technical section submission is still under review. However, CVM finds the gap in safety information submitted to be acceptable to support approval.	194
Efficacy Technical Section	Pfizer	7/27/2007	Submission of summary of the marketing experience including European Adverse Event Reports relating to the Effectiveness Technical Section (EFF TS).	
Efficacy Technical Section	CVM G-0234-OT	11/13/2007	CVM reviewed and commented on the data provided for the post-marketing experience in Europe for the first 11 months following the European approval of Cefovecin.	109

Efficacy Technical Section	Pfizer	6/29/2007	Submission of a response to CVMs incomplete SI EFF TS incomplete letter dated 14 July 2006 and subsequent conferences on 14 September 2006 and 17 May 2007 to resolve all remaining issues pertaining to cefovecin EFF TS for the treatment of skin infections (SI) in dogs.	
Efficacy Technical Section	CVM/ P-0233-EF	1/14/2008	CVM considers the Effectiveness Technical Section for cefovecin proposed for the treatment of skin infections in dogs to be complete.	199
CMC				
CMC	Pfizer	5/1/2002	Request Protocol Review of the Drug Substance VICH Stability Program.	
CMC	CVM E0016	6/5/2002	CVM reviewed and comments on the Stability protocols for the Drug Substance and the Drug Product.	35
CMC	Pfizer	5/27/2003	Request review of the Sterile Process Validation Package Protocol.	
CMC	CVM E0189	8/1/2003	CVM provides comments on the Sterile Process Validation Package Protocol which was submitted 27 May 2003	66

CMC	Pfizer	4/16/2004	Submission of the CHEMISTRY, MANUFACTURING and CONTROL Technical Section including draft labeling...	
CMC	CVM P0195	10/15/2004	CVM finds CMC Technical Section to be incomplete	182
CMC	Pfizer	3/29/2005	Submission of an amendment to the CMC TS in response to CVM's Incomplete Letter dated 15Oct2004.	
CMC	CVM P-0202	11/3/2005	CVM completes Chemistry, Manufacturing, Control Procedures Technical Section	219
Environmental Assessment				
Environmental Assessment	Pfizer	12/22/1999	Pfizer requests categorical exclusion from the requirement to prepare an environmental assessment for 10-612.	
Environmental Assessment	CVM E0003, G0004	2/28/2000	CVM agrees that a categorical exclusion is appropriate.	68
Environmental Assessment	Pfizer	10/29/2003	Submission requesting categorical exclusion from the requirement to prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) for the INAD and NADA	
Environmental Assessment	CVM G0193	11/26/2003	CVM grants categorical exclusion and considers Environmental Technical Section complete.	28

All Other Information				
AOI	Pfizer	12/7/2007	Submission of All Other Information Technical Section	
AOI	CVM M-0235-AO	3/12/2008	CVM considers the All Other Information Technical Section for Convenia in dogs, to be complete	96
Labeling				
Labeling	Pfizer	12/10/2007	Submission of Label Technical Section	
Labeling	CVM M-0236-LB	3/6/2008	CVM considers Labeling Technical Section to be complete.	87
Administrative NADA				
NADA	Pfizer	3/15/2008	Submission of original administrative NADA	
NADA	CVM 141-285-A-0000-OT	4/25/2008	CVM approves an original new animal drug application (NADA) for Convenia for skin infections in dogs	41

EXHIBIT F

Chronology of Review of CONVENIA (Cefovecin sodium) File for Cats (INAD 10-613:

File Section	Source	Date	Description	Time Under Review (Days)
INAD				
INAD cat	Pfizer	8/6/1999	Original request to open INAD for cat	
INAD cat	CVM A0000	11/16/1999	CVM assigned file numbers for INAD	102
Safety				
Safety (Toleration)	Pfizer	11/10/1999	Request review of drug tolerance protocol 1481N-60-99-216 entitled "Acute Safety Toleration".	
Safety (Toleration)	CVM E0002	1/21/2000	CVM finds the Acute Safety Toleration Protocol acceptable as submitted making revisions (listed). A final protocol will need to be submitted for file.	72
Safety (Toleration)	Pfizer	5/10/2000	Submission of revised protocol 1481N-60-99-216 entitled Acute Safety Toleration of UK-287,074 in cats.	
Safety (Toleration)	CVM E0006	7/24/2000	CVM finds the Final Revised target Animal Safety Drug Tolerance protocol acceptable	75

Safety (MOS)	Pfizer	3/15/2000	Submission for review of Margin of Safety protocol (1482N-60-99-210).	
Safety (MOS)	CVM E0005	5/22/2000	Concurrence on Margin of Safety protocol (1482N-60-99-210) with suggested CVM changes.	68
Safety (MOS)	Pfizer	5/22/2000	Request review of "Exploratory Margin of Safety and Injection Site Toleration" study 1482N-60-00-221	
Safety (MOS)	CVM E0007	7/31/2000	CVM finds the Exploratory MOS and Injection Site Toleration Protocol (1461N-60-00-419) acceptable as submitted and makes several suggested revisions.	70
Safety (Stat Analysis)	Pfizer	12/11/2000	Request review and comments on the statistical approach for evaluating clinical pathology variables assoc. w/ TAS studies.	
Safety (Stat Analysis)	CVM A0000	8/30/2001	CVM has reviewed the Statistical Approach for evaluating clinical pathology variables associated w/ TAS studies (as submitted 12/11/2000) and has requested (listed) information be provided in support.	262

Safety (Technical Section)	Pfizer	8/30/2002	Submission of TAS Technical Section for review	
Safety (Technical Section)	CVM/ P0182	4/1/2003	CVM considers the Target Animal Safety Technical Section to be complete for the purpose of recommending approval of a New Animal Drug Application. Comments added.	214
Safety (Technical Section)	Pfizer	7/1/2003	Submission of a response to CVM's comments dated 01 April 2003 on the Target Animal Safety Technical Section	
Safety (Technical Section)	CVM G0202	10/17/2003	CVM acknowledges response of July 1, 2003.	108
Safety (Technical Section)	Pfizer	10/29/2004	Submission of further response to April 1, 2003 review of TAS, including an amendment to an error noted in the statistical evaluation for the Margin of Safety study.	
Safety (Technical Section)	CVM G-0210	1/28/2005	CVM provides a copy of the FOI Summary reflecting the requested changes in the safety section in statistical significance for injection site swellings identified in the study.	91

Efficacy				
Efficacy Clinical Field Trial Protocol	Pfizer	12/22/2000	Request review of the Efficacy and Safety of UK287,074 in the treatment of SST in Cats Protocol 1183C-60-00-237	
Efficacy Clinical Field Trial Protocol	CVM E0012	4/5/2001	CVM finds the Clinical Field Effectiveness Protocols for SST infections not acceptable and lists issues requiring revisions.	104
Efficacy Clinical Field Trial Protocol	Pfizer	6/14/2001	Request review of the Revised SST Pivotal Clinical Trial Protocol 1183C-60-00-237.	
Efficacy Clinical Field Trial Protocol	CVM E0018	8/16/2001	CVM finds the SST Efficacy and Safety Protocols acceptable with their (listed) clarifications	63
Efficacy Technical Section	Pfizer	10/7/2003	Submission of Effectiveness Technical Section for Skin Infections in Cats.	
Efficacy Technical Section	CVM/ P0203, T0205	12/31/2003	CVM incompletes the Effectiveness Technical Section (SSTI Claim)	85
Efficacy Technical Section	Pfizer	4/21/2004	Submission of an interim update regarding the microbiological data and a proposal to resolve issues	
Efficacy Technical Section	CVM G-0207	8/4/2004	CVM has reviewed our submissions and finds the proposed plan of action to be reasonable.	105

Efficacy Technical Section	Pfizer	12/20/2004	Submission of an Amendment to the Effectiveness Technical Section	
Efficacy Technical Section	CVM P0211	8/24/2005	CVM incompletes the Effectiveness Technical Section (EFF TS) for SSTI (soft skin tissue infections) and attaches Draft FOI and Labeling.	247
Efficacy Technical Section	Pfizer	12/7/2005	Submission of a response to CVMs Incomplete Letter dated 24 August 2005	
Efficacy Technical Section	CVM P-0218	7/14/2006	CVM Incompletes the Skin Infection (SI) Effectiveness Technical Section submitted on 07 December 2005 for the treatment of skin infections	219
Efficacy Technical Section	Pfizer	5/11/2007	Submission of a response to the gap in safety data for the Effectiveness Technical Section for the treatment of skin infections to resolve issues pertaining to the gap in safety data (Days 42-650 in clinical trials	
Efficacy Technical Section	CVM P-0227-EF	11/21/2007	CVM incompletes 11 May 2007 submission because an effectiveness technical section submission is still under. However, CVM finds the gap in safety information submitted t o be acceptable to support approval.	194

Efficacy Technical Section	Pfizer	6/29/2007	Submission of a response to CVMs incomplete SI EFF TS incomplete letter dated 14 July 2006 and subsequent conferences on 14 September 2006 and 17 May 2007 to resolve all remaining issues pertaining to cefovecin EFF TS for the treatment of skin infections (SI) in cats.	
Efficacy Technical Section	CVM/ P-0228-EF	1/14/2008	CVM considers the Effectiveness Technical Section for cefovecin proposed for the treatment of skin infections (wounds and abscesses) caused by susceptible strains of *Pasteurella multocida* in cats to be complete.	199
Efficacy Technical Section	Pfizer	7/27/2007	Submission of a document summarizing the marketing experience including European Adverse Event Reports relating to the Effectiveness Technical Section (EFF TS) being submitted under general correspondence as discussed with CVM	
Efficacy Technical Section	CVM G-0229-OT	11/13/2007	CVM reviewed and commented on the data provided for the post-marketing experience in Europe for the first 11 months following the European approval of Cefovecin.	109

CMC				
CMC	Pfizer	5/1/2002	Request Protocol Review of the Drug Substance VICH Stability Program.	
CMC	CVM E0016	6/5/2002	CVM reviewed and comments on the Stability protocols for the Drug Substance and the Drug Product.	35
CMC	Pfizer	5/27/2003	Request review of the Sterile Process Validation Package Protocol.	
CMC	CVM E0201	8/1/2003	CVM provides comments on the Sterile Process Validation Package Protocol which was submitted 27 May 2003	66
CMC	Pfizer	4/16/2004	Submission of the CHEMISTRY, MANUFACTURING and CONTROL Technical Section including draft labeling.	
CMC	CVM P0206	10/15/2004	CVM finds CMC Technical Section to be incomplete	182
CMC	Pfizer	3/29/2005	Submission of an amendment to the CMC TS in response to CVM's Incomplete Letter dated 15Oct2004.	
CMC	CVM P-0212	11/3/2005	CVM completes Chemistry, Manufacturing, Control Procedures Technical Section	219

Environmental Assessment				
Environmental Assessment	Pfizer	12/22/1999	Pfizer requests categorical exclusion from the requirement to prepare an environmental assessment for 10-612.	
Environmental Assessment	CVM E0003, G0004	2/28/2000	CVM agrees that a categorical exclusion is appropriate.	68
Environmental Assessment	Pfizer	10/29/2003	Submission requesting categorical exclusion from the requirement to prepare an Environmental Assessment (EA) or Environmental Impact Statement (EIS) for the INAD and NADA	
Environmental Assessment	CVM G0204	11/26/2003	CVM grants categorical exclusion and considers Environmental Technical Section complete.	28
All Other Information				
AOI	Pfizer	12/7/2007	Submission of All Other Information Technical Section	
AOI	CVM M-0230-AO	3/12/2008	CVM considers the All Other Information Technical Section for Convenia in dogs, to be complete	96

Labeling				
Labeling	Pfizer	12/10/2007	Submission of Label Technical Section	
Labeling	CVM M-0231-LB	3/6/2008	CVM considers Labeling Technical Section to be complete.	87
Administrative NADA				
NADA	Pfizer	3/15/2008	Submission of original administrative NADA	
NADA	CVM 141-285-A-0000-OT	4/25/2008	CVM approves an original new animal drug application (NADA) for Convenia for skin infections in dogs	41

EXHIBIT G

Patent Assignment Abstract of Title

Total Assignments: 2

Application #: 07934667 **Filing Dt:** 01/22/1993 **Patent #:** NONE **Issue Dt:**

PCT #: NONE **Publication #:** NONE **Pub Dt:**

Inventors: JOHN H. BATESON, GEORGE BURTON, STEPHEN C.M. FELL

Title: CEPHALOSPORINS AND HOMOLOGUES, PREPARATIONS AND PHARMACEUTICAL COMPOSITIONS

Assignment: 1

Reel/Frame: 006481 / 0984 **Received:** **Recorded:** 04/09/1993 **Mailed:** 06/01/1993 **Pages:** 2

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST.

Assignors: BATESON, JOHN HARGREAVES **Exec Dt:** 01/06/1993

BURTON, GEORGE **Exec Dt:** 01/06/1993

FELL, STEPHEN CHRISTOPHER MARTIN **Exec Dt:** 01/06/1993

Assignee: BEECHAM GROUP P.L.C.
FOUR NEW HORIZONS COURT
HARLEQUIN AVENUE, BRENTFORD
MIDDLESEX TW8 9EP, ENGLAND

orrespondent: JANICE E. WILLIAMS
SMITHKLINE BEECHAM CORPORATION
CORPORATE PATENTS - UW2220
P.O. BOX 1539
KING OF PRUSSIA, PA 19406-0939

ssignment: 2

Reel/Frame: 008447 / 0046 **Received:** 04/18/1997 **Recorded:** 01/02/1997 **Mailed:** 08/01/1997 **Pages:** 8

Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).

Assignor: BEECHAM GROUP P.L.C. **Exec Dt:** 07/24/1996

Assignee: PFIZER INC.
235 EAST 42ND STREET
NEW YORK, NEW YORK 10017

orrespondent: LADAS & PARRY
LANNING G. BRYER
26 WEST 61ST STREET
NEW YORK, NY 10023

Search Results as of: 05/22/2008 12:11 PI

DOCKET NO. B3025

Form PTO-1595
1-31-92

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

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PATENTS ONLY

To the Honorable Commissioner of Patents and Trademarks, Please record the attached original documents or copy thereof.

1. Name of conveying party(ies)

John Hargreaves Bateson
George Burton
Stephen Christopher Martin Fell

Additional names of conveying party(ies) attached?
[ ] yes [X] no

2. Name and address of receiving party(ies)

Beecham Group p.l.c.
Four New Horizons Court
Harlequin Avenue, Brentford,
Middlesex TW8 9EP, England

Additional name(s) and addresses attached?
[ ] yes [X] no

3. Description of the interest conveyed:

[X] Assignment, please record and return
[ ] Security Agreement
[ ] Other
[ ] Merger
[ ] Change of Name

Execution Date 16-9

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93 APR 19 AM 10 16

4. Application number(s) or patent number(s). Additional Sheets attached? _ Yes X No

A. Patent Application No.(s)
U.S. Serial No. 07/934,667

B. Patent No.(s)

If this document is being filed together with a new application, the execution date of the application is

5. Name and address of party to whom correspondence concerning documents should be mailed:

Janice E. Williams
SmithKline Beecham Corporation
Corporate Patents - UW2220
P.O. Box 1539
King of Prussia, PA 19406-0939

6. Total number of applications and patents involved 1.

7. Total Fee (37 C.F.R. 3.41) $ 40.00

8. Please charge this fee to deposit account No. 19-2570.
The Commissioner is hereby authorized to charge any additional fees under 37 CFR 1.16 or 1.17 which may be required by this paper, or credit any overpayment, to our Deposit Account No. 19-2570.
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SC13137 04/15/93 07934667 19-2570 130 581 40.00CH

9. Statement and Signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Janice E. Williams
Name of Person signing

Janice E. Williams
Signature

April 6, 1993
Date

Total number of pages comprising cover sheet 3

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REEL 6491 FRAME 0984

U.S. Convention

US/3

ASSIGNMENT

WHEREAS WE, John Hargreaves BATESON, George BURTON and Stephen Christopher Martin FELL
of: SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ, England

have made an invention entitled: NOVEL COMPOUNDS for which on even date we executed an application for Letters Patent of the United States of America;

NOW, THEREFORE, in consideration of One Pound Sterling and other valuable consideration paid to us by Beecham Group p.l.c., (hereinafter "ASSIGNEE"), the receipt of which is hereby acknowledged, and intending to be legally bound, we do hereby assign unto the said ASSIGNEE, its successors and assigns, the entire right, title and interest in and to the said invention, said executed application, any division, continuation and continuation-in-part of said application and reissue applications, and all Letters Patents of the United States of America to be obtained therefor;

We hereby covenant that no assignment, sale, agreement or encumbrance has been or will be made or entered into which would conflict with this assignment and sale;

In addition we agree to provide ASSIGNEE upon its request with all pertinent facts and documents relating to said invention, and said Letters Patent as may be known and accessible to us, and to testify as to the same in any interference or litigation related thereto, and to execute further instruments (including divisional, continuation, continuation-in-part or reissue applications, affidavits or other instruments) required to apply for, obtain, maintain and enforce said application and said Letters Patent which may be necessary; this agreement to be binding upon our heirs, executors and administrators.

Inventor I

John Hargreaves Bateson

John Hargreaves BATESON

Date: 6th January 1993

Inventor II

George BURTON

Date: 6th January 1993

Inventor III

Stephen Christopher Martin Fell

Stephen Christopher Martin FELL

Date: 6th January 1993

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APR -9 93

REEL 6481 FRAME 0985

FORM PTO-1595 (Rev. 6-93)
OMB No. 0651-0011 (exp. 4/94)

1-2-97 MRD 581 1,120

RECORD.

04-18-1997

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

JAN 02 1997

Tab settings ⇨ ⇨ ⇨ ▼ ▼

To the Honorable Commissioner of Patents and documents or copy thereof.

100358201

1. Name of conveying party(ies): MRD 1-2-97

BEECHAM GROUP p.l.c.

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies) 96/45/68

Name: PFIZER INC.

Internal Address:

Street Address: 235 EAST 42nd STREET

City: NEW YORK State: NEW YORK ZIP: 10017-5755

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other

Execution Date: JULY 24, 1996 and DECEMBER 4, 1996

4. Application number(s) or patent number(s):

If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)

(See Schedule)

B. Patent No.(s)

(See Schedule)

Additional numbers attached? ☒ Yes ☐ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: LANNING G. BRYER, ESQ.

Internal Address: LADAS & PARRY

Street Address: 26 WEST 61st STREET

City: NEW YORK State: N.Y. ZIP: 10023

6. Total number of applications and patents involved: 28

7. Total fee (37 CFR 3.41).....$ 1,120.00

☒ Enclosed

☐ Authorized to be charged to deposit account

8. Deposit account number: NA

(Attach duplicate copy of this page if paying by deposit account)

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

01/02/1997 [illegible] 00000010 08358201
01 FC:481 1,120.00 CK

LANNING G. BRYER
Name of Person Signing

Signature

DECEMBER 27, 1996
Date

(Our Ref. 95 GP 05-10-47 (H) Total number of pages including cover sheet, attachments, and document: 35

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

PATENT
REEL: 8358 FRAME: 0046

UNITED STATES OF AMERICA

S C H E D U L E

PATENT NOS.
RE31301
4164568
4401674
4661489
4707474
4866056
4876086
4879287
5008248
5055596
5064649
5134131
5158946
5246926
5275816

DESIGN REGISTRATION NO.
321759

PATENT APPLICATION NOS.	FILING DATE
08/064080	November 11, 1991
798971	November 29, 1991
890700	May 29, 1992
933922	August 24, 1992
934667	January 22, 1993
971851	January 22, 1993
987276	March 9, 1993
108790	August 18, 1993
08/064014	January 7, 1994
08/221656	April 1, 1994
08/303889	September 9, 1994
940285	December 11, 1986

[redacted]

UNITED STATES OF AMERICA

A S S I G N M E N T

WHEREAS, **BEECHAM GROUP p.l.c.**, a corporation organized under the laws of England, with an office at Four New Horizons Court, Harlequin Avenue, Brentford, Middlesex TW8 9EP, England, (hereinafter called the ASSIGNOR) is the proprietor of the Patent[s], Design Registration[s] and Patent Application[s] in the United States of America:

(Set forth on the attached schedule)

AND WHEREAS, **PFIZER INC.**, a corporation organized and existing under the laws of the State of Delaware, United States of America, located at 235 East 42nd Street, New York, New York 10017-5755, United States of America, (hereinafter called the ASSIGNEE) is desirous of acquiring the entire right, title and interest of the ASSIGNOR in and to the aforesaid Patent[s], Design Registration[s] and Patent Application[s],

NOW, THEREFORE, in consideration of U.S. $1.00 and other good and valuable consideration, the ASSIGNOR hereby assigns to the ASSIGNEE, its successors and assigns, absolutely all its right, title and interest in and to the aforesaid Patent[s], Design Registration[s] and Patent Application[s] including all emoluments, advantages, profits and benefits accruing or belonging thereto.

The ASSIGNOR consents to the ASSIGNEE'S proceeding with the aforesaid Patent Application[s], if any, in its own name in substitution of the ASSIGNOR and the said ASSIGNOR agrees to do all that may be required to put the foregoing into effect.

Agreed to: _____ [Signature]
for and on behalf of **BEECHAM GROUP p.l.c.**

ATTORNEY
[Title]

24th July 1996
[Date]

IN WITNESS WHEREOF, the said ASSIGNEE has executed this document and affixed its corporate seal this 4th day of December 1996.

PFIZER INC.

[Corporate Seal]

By: _____ [Title]
Peter C. Richardson
Assistant Secretary

Attest:
_____ [Title]
Paralegal

[See over for Statutory Declaration and Notarial Acknowledgment]

[redacted]

STATUTORY DECLARATION

I, David Roberts residing at SmithKline Beecham, SB House, Great West Road, Brentford, Middlesex TW8 9BD, England

do solemnly and sincerely declare as follows:

I am ATTORNEY of BEECHAM GROUP p.l.c., a corporation organized under the laws of England, with an office at Four New Horizons Court, Harlequin Avenue, Brentford, Middlesex TW8 9EP, England, and I am duly authorized to sign the foregoing instrument in the name of said corporation and that the purposes for which said instrument are granted are within the scope of the objectives or activities of said corporation.

AND I MAKE THIS SOLEMN DECLARATION conscientiously believing it to be true and by virtue of the Statutory Declaration Act 1835.

David Roberts

Declared before me at Middlesex, England

this 24th day of July , 1996.

[signature of solicitor]

[redacted]

L&P - 52 - Single Ack. [86/VIII]

CORPORATE ACKNOWLEDGMENT

UNITED STATES OF AMERICA]
]
STATE OF NEW YORK] SS:
]
COUNTY OF NEW YORK]

On this 4th. day of December 19 96 , before me personally appeared PETER C. RICHARDSON to me known, who, being by me duly sworn, did depose and say that he/~~she~~ is the Assistant Secretary of the corporation described in and which executed the foregoing instrument; that he/~~she~~ knows the seal of said corporation; that the said seal affixed to said instrument is such corporate seal; that it was so affixed by order of the Board of Directors of said corporation and that he/~~she~~ signed his/~~her~~ name thereto by like order.

Oswaldo F. Lemos
Notary Public

[illegible] New York
[illegible] York County
Commission Expires Apr 30, 1997

[Seal]

[redacted]

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

MARCH 06, 1997

PTAS

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LADAS & PARRY
LANNING G. BRYER
26 WEST 61ST STREET
NEW YORK, NY 10023

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, NORTH TOWER BUILDING, SUITE 10C35, WASHINGTON, D.C. 20231.

RECORDATION DATE: 01/02/1997

REEL/FRAME: 8290/0037
NUMBER OF PAGES: 5

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
BEECHAM GROUP, P.L.C.

DOC DATE: 07/24/1996

ASSIGNEE:
PFIZER, INC.
235 EAST 42ND STREET
NEW YORK, NEW YORK 10017-5755

SERIAL NUMBER: 08064080
PATENT NUMBER:

FILING DATE: 01/07/1994
ISSUE DATE:

SERIAL NUMBER: 08798971
PATENT NUMBER:

FILING DATE:
ISSUE DATE:

SERIAL NUMBER: 08890700
PATENT NUMBER:

FILING DATE:
ISSUE DATE:

SERIAL NUMBER: 08933922
PATENT NUMBER:

FILING DATE:
ISSUE DATE:

SERIAL NUMBER: 08934667 PATENT NUMBER:	FILING DATE: ISSUE DATE:
SERIAL NUMBER: 08971851 PATENT NUMBER:	FILING DATE: ISSUE DATE:
SERIAL NUMBER: 08987276 PATENT NUMBER:	FILING DATE: ISSUE DATE:
SERIAL NUMBER: 08108790 PATENT NUMBER:	FILING DATE: 08/18/1993 ISSUE DATE:
SERIAL NUMBER: 08064014 PATENT NUMBER:	FILING DATE: 01/07/1994 ISSUE DATE:
SERIAL NUMBER: 08221656 PATENT NUMBER:	FILING DATE: 04/01/1994 ISSUE DATE:
SERIAL NUMBER: 08303889 PATENT NUMBER:	FILING DATE: 09/09/1994 ISSUE DATE:
SERIAL NUMBER: 08940285 PATENT NUMBER:	FILING DATE: ISSUE DATE:
SERIAL NUMBER: 06345474 PATENT NUMBER: RE31301	FILING DATE: 02/03/1982 ISSUE DATE: 07/05/1983
SERIAL NUMBER: 05776536 PATENT NUMBER: 4164568	FILING DATE: 03/11/1977 ISSUE DATE: 08/14/1979
SERIAL NUMBER: 06334437 PATENT NUMBER: 4401674	FILING DATE: 12/24/1981 ISSUE DATE: 08/30/1983
SERIAL NUMBER: 06630524 PATENT NUMBER: 4661489	FILING DATE: 07/13/1984 ISSUE DATE: 04/28/1987
SERIAL NUMBER: 06492472 PATENT NUMBER: 4707474	FILING DATE: 05/06/1983 ISSUE DATE: 11/17/1987
SERIAL NUMBER: 06908379 PATENT NUMBER: 4866056	FILING DATE: 09/17/1986 ISSUE DATE: 09/12/1989
SERIAL NUMBER: 07098326 PATENT NUMBER: 4876086	FILING DATE: 09/17/1987 ISSUE DATE: 10/24/1989
SERIAL NUMBER: 07288023 PATENT NUMBER: 4879287	FILING DATE: 12/21/1988 ISSUE DATE: 11/07/1989
SERIAL NUMBER: 07474635 PATENT NUMBER: 5008248	FILING DATE: 01/30/1990 ISSUE DATE: 04/16/1991
SERIAL NUMBER: 07559989 PATENT NUMBER: 5055596	FILING DATE: 07/30/1990 ISSUE DATE: 10/08/1991

SERIAL NUMBER: 07407231 PATENT NUMBER: 5064649	FILING DATE: 09/14/1989 ISSUE DATE: 11/12/1991
SERIAL NUMBER: 07365239 PATENT NUMBER: 5134131	FILING DATE: 06/12/1989 ISSUE DATE: 07/28/1992
SERIAL NUMBER: 07583226 PATENT NUMBER: 5158946	FILING DATE: 09/14/1990 ISSUE DATE: 10/27/1992
SERIAL NUMBER: 07500397 PATENT NUMBER: 5246926	FILING DATE: 03/28/1990 ISSUE DATE: 09/21/1993
SERIAL NUMBER: 07578662 PATENT NUMBER: 5275816	FILING DATE: 09/04/1990 ISSUE DATE: 01/04/1994

TARA WASHINGTON, EXAMINER
ASSIGNMENT DIVISION
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